PRELIMINARY SCREENING OF PROTEINACEOUS AND GLYCEROL-RELATED COMPOUNDS IN COMMERCIAL CIGARETTE FILTERS: IMPLICATIONS FOR HALAL CRITICAL POINT ASSESSMENT

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Abstract: Cigarette filters are generally regarded as inert components composed primarily of cellulose acetate fibers and plasticizers such as triacetin. However, limited independent analytical studies have investigated the presence of additional compounds that may be incorporated during manufacturing. From a halal science perspective, the presence of proteinaceous materials or glycerol-related compounds may represent a potential halal critical point because their permissibility depends on their biological origin and processing method. This study conducted a preliminary screening of proteinaceous and glycerol-related compounds in several commercial cigarette filters using chromatographic and spectrometric analytical approaches. In addition, a hemoglobin-equivalent colorimetric assay was applied as an initial indicator of protein-like substances. The results indicated the presence of glycerol-related compounds consistent with commonly used plasticizers and humectants in cigarette filter manufacturing. The preliminary screening also suggested the presence of proteinaceous signals in certain samples, although the analytical approach used in this study does not allow for the confirmation of the species origin. These findings highlight the need for more comprehensive analytical verification, including advanced proteomic or DNA-based techniques, to determine the source of such compounds. The study underscores the importance of transparency in material sourcing and manufacturing processes for consumer products that may involve animal-derived substances. Furthermore, this research contributes to the development of analytical frameworks for identifying potential halal critical points in non-food consumer products such as tobacco-related materials.

Keywords: *acrolein; Bradford; cigarette filters; glycerol; hemoglobin; protein*

Abstrak: Filter rokok umumnya dianggap sebagai komponen inert yang terutama tersusun dari serat selulosa asetat dan plasticizer seperti triacetin. Namun demikian, kajian analitik independen yang meneliti keberadaan senyawa tambahan yang mungkin dimasukkan selama proses manufaktur masih sangat terbatas. Berdasarkan perspektif sains halal, keberadaan bahan bersifat protein (proteinaceous materials) atau senyawa yang berkaitan dengan gliserol berpotensi menjadi titik kritis halal, karena kehalalannya

bergantung pada asal biologis serta metode pengolahannya. Penelitian ini melakukan skrining awal terhadap keberadaan senyawa proteinaceous dan senyawa terkait gliserol pada beberapa filter rokok komersial dengan menggunakan pendekatan analisis kromatografi dan spektrometri. Selain itu, uji kolorimetri hemoglobin-ekuivalen digunakan sebagai indikator awal untuk mendeteksi keberadaan zat yang menyerupai protein. Hasil penelitian menunjukkan adanya senyawa terkait gliserol yang konsisten dengan penggunaan *plastisizer* dan humektan yang umum digunakan dalam pembuatan filter rokok. Skrining awal juga menunjukkan adanya sinyal yang mengindikasikan keberadaan senyawa proteinaceous pada beberapa sampel, meskipun pendekatan analitik yang digunakan dalam penelitian ini belum memungkinkan konfirmasi asal spesiesnya. Temuan ini menegaskan perlunya verifikasi analitik yang lebih komprehensif, termasuk penggunaan teknik proteomik tingkat lanjut atau metode berbasis DNA, untuk menentukan sumber senyawa tersebut secara lebih pasti. Penelitian ini juga menekankan pentingnya transparansi dalam sumber bahan baku dan proses manufaktur pada produk konsumen yang berpotensi melibatkan bahan turunan hewani. Selain itu, penelitian ini berkontribusi dalam pengembangan kerangka analitik untuk mengidentifikasi potensi titik kritis halal pada produk konsumen nonpangan, seperti material yang berkaitan dengan produk tembakau.

Kata kunci: akrolein; Bradford; filter rokok; gliserol; hemoglobin; protein

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Introduction

Cigarette consumption remains a major global public health concern. It is estimated that more than 5 trillion cigarettes are consumed annually worldwide, contributing substantially to preventable morbidity and mortality (World Health Organization, 2023). Indonesia ranks among the countries with the highest smoking prevalence, particularly among adult males, with rates exceeding 60% in some demographic groups (GBD 2021 Tobacco Collaborators, 2023). While extensive research has focused on toxicants generated during tobacco combustion and their associated health risks, comparatively less attention has been directed toward the chemical composition of cigarette filter materials themselves.

Modern cigarette filters are primarily composed of cellulose acetate fibers, a semi-synthetic polymer derived from cellulose, which are bonded using plasticizers such as triacetin (glycerol triacetate) to form and maintain the structural integrity of the filter rod (Zhang et al., 2020; D'Urso et al., 2024; Novotny & Hamzai, 2024).

In addition to these principal components, modern cigarette filters may incorporate additional materials to enhance performance, including flavor capsules embedded in the filter, humectants such as glycerol and propylene glycol that regulate moisture and modify smoke properties, and other additives designed to improve sensory characteristics (Kassem et al., 2024; Kyriakos et al., 2023; Mus et al., 2024; Xizheng et al., 2018). However, detailed disclosure of specific additive

compositions in commercially available cigarette filters is generally limited, and independent verification studies remain scarce

From an analytical chemistry perspective, the majority of tobacco-related investigations have centered on mainstream smoke constituents—such as tar, nicotine, carbon monoxide, tobacco-specific nitrosamines (TSNAs), and volatile organic compounds—rather than the intrinsic composition of the filter matrix (Pennings et al., 2020; Setshedi et al., 2025).

Nevertheless, the filter should not be regarded solely as an inert physical barrier. Instead, it may act as a carrier or reservoir for intentionally added substances introduced during manufacturing. Patent literature has described the potential incorporation of adsorption-enhancing agents, including porphyrin-based complexes and other reactive materials designed to bind carbon monoxide and nitrogen oxides (Stavridis & Deliconstantinos, 1994; Na et al., 2011). Despite these reports, peer-reviewed analytical confirmation of such compounds in commercially marketed cigarette filters remains limited.

In parallel with advances in tobacco product chemistry, halal authentication has emerged as an important interdisciplinary field, particularly in Muslim-majority countries. Halal compliance extends beyond food products to include pharmaceuticals, cosmetics, and consumer goods when animal-derived materials or critical processing steps are involved (Magfiratun & Awaludin, 2024; Rahmah & Barizah, 2020). From a halal science perspective, certain chemical compounds such as glycerol and protein-based materials are not inherently permissible or impermissible; rather, their status depends on their biological origin and processing methods. Glycerol, for example, may be produced through multiple routes, including petrochemical synthesis, transesterification or hydrolysis of plant oils and animal fats, and microbial fermentation, with each production pathway potentially carrying different compositional and regulatory implications (Kazimierowicz et al., 2024; Kaur et al., 2020). Similarly, proteinaceous substances may originate from plant, microbial, or animal sources, necessitating analytical verification when source transparency is lacking.

Despite the rapid development of analytical techniques for halal authentication, including chromatographic methods, mass spectrometry, immunoassays, and DNA-based approaches, most studies have focused on food and pharmaceutical products, with limited attention given to non-food matrices such as tobacco products (Rohman et al., 2020; Nurani et al., 2022; Rohman & Windarsih, 2020; Yusop & Bakar, 2020).

In particular, empirical data concerning the possible presence of proteinaceous or glycerol-related substances in commercial cigarette filters marketed in Indonesia remain limited. This gap creates uncertainty in identifying potential halal critical points associated with additive sourcing and manufacturing transparency.

Therefore, this study aimed to perform a preliminary analytical screening of selected commercial cigarette filters to detect (1) soluble protein-like compounds, (2) heme-reactive chromophores, and (3) glycerol-related compounds. The objective was not to confirm a specific biological origin, but rather to identify potential chemical indicators that may warrant further investigation using advanced analytical techniques. By integrating analytical chemistry with halal critical point assessment, this research seeks to provide an initial scientific basis for evaluating additive transparency in tobacco filter materials.

Materials and Methods

Study Design

This study employed a laboratory-based experimental design to perform a preliminary chemical screening of proteinaceous compounds and glycerol-related substances in commercial cigarette filters. All analyses were conducted in triplicate to ensure reproducibility. The study focused on a comparative evaluation between locally produced and imported cigarette brands available in the Indonesian market**.**

Sample Collection

A total of thirteen commercially available cigarette brands were purchased from authorized retailers in Jakarta, Indonesia, in 2016. Six brands were locally manufactured (coded L1 to L6) and seven were imported products (coded I1 to I7). For each brand, three different production batches were obtained to minimize batch variability.

From each pack, five cigarette filters were randomly selected and pooled to form one composite sample. All filters were manually separated from tobacco rods using sterile stainless-steel scissors and stored in sterile polypropylene containers at room temperature prior to analysis.

The materials used in this study included cellulose acetate fibers from commercial filters, standard protein, ammonium sulfate, dialysis membranes, and Bovine Serum Albumin (BSA) for analytical standards.

Reagents and Chemicals

The following analytical-grade reagents were used: Coomassie Brilliant Blue G-250 (Sigma-Aldrich (Merck)), Bovine Serum Albumin (BSA) standard (≥98% purity) (Sigma-Aldrich (Merck)), Drabkin's reagent (potassium ferricyanide, potassium cyanide, potassium dihydrogen phosphate) (Sigma-Aldrich (Merck)), hydrochloric acid (HCl, 1 M) (*Merck KGaA* (Germany), sodium hydroxide (NaOH, 1 M) (Fisher Scientific), and distilled deionized water (18.2 MΩ·cm) (Merck Millipore). All reagents were obtained from certified chemical suppliers and used without further purification.

Preparation of Filter Extracts

Approximately 0.500 ± 0.005 g of shredded filter material was accurately weighed using an analytical balance. The sample was transferred into a 50 mL centrifuge tube and extracted with 10 mL of distilled deionized water.

Extraction was performed using vortex mixing (2 minutes), sonication (40 kHz, 30 minutes, room temperature), and centrifugation (5000 rpm, 15 minutes).

The supernatant was collected and filtered through a 0.45 µm membrane filter prior to analysis. Blank extraction (without filter material) was performed under identical conditions as a negative control.

Determination of Total Protein Content (Bradford Assay)

Total soluble protein was determined using the Bradford colorimetric method.

Standard Curve Preparation

BSA standards were prepared at concentrations of: 0, 40, 80, 120, 160, and 200 µg/mL. A calibration curve was constructed by plotting absorbance versus protein concentration.

Assay Procedure

200 µL of sample extract was mixed with 1.0 mL Bradford reagent. The mixture was incubated at room temperature for 10 minutes. Absorbance was measured at 615 nm using a UV–Vis spectrophotometer. All measurements were performed in triplicate. Protein concentration was calculated from the calibration curve and expressed as µg protein per gram of filter material (µg/g).

Preparation of Positive Control.

The standard filter was dipped into a solution of BSA in MES buffer at concentrations of 100, 200, 300, 400, and 500 ppm, then incubated for 3 hours. The filter was dried in an oven at 50° C until completely dry. After drying, the filter was cut into thin slices, dissolved in 5 mL of distilled water, and incubated for 3 hours. A 100 µL of the solution was transferred into a test tube, followed by the addition of 5 mL of Bradford reagent. The tubes were covered with aluminum foil, shaken, and incubated at room temperature for 5 minutes. The absorbance of each solution was measured using a spectrophotometer at a wavelength of 615 nm.

Protein Measurement in Cigarette Samples.

Protein analysis was conducted separately for the cigarette paper, tobacco mixture (tobacco, cloves, and other components), and the filter. The filter was cut into thin slices, dissolved in 5 mL of distilled water, and incubated for 3 hours. 100 mL of the solution is taken and then put into a test tube, and 5 mL of Bradford reagent was added. Furthermore, the tube was covered with aluminum foil, shaken, incubated at room temperature for 5 minutes, and then measured. The absorbance was read using a spectrophotometer at a wavelength of 615 nm.

Qualitative Screening of Glycerol-Related Compounds

Preparation of Control Filters Containing Glycerol.

A standard cellulose acetate filter was injected with 0, 100, 200, 300, 400, and 500 mL of glycerol. The filters were incubated in an oven at 50 °C until dry.

Preliminary qualitative detection of glycerol-related substances was performed using an acid dehydration reaction: 1 mL of extract was mixed with 1 mL concentrated HCl. The mixture was heated at 100°C for 10 minutes in a water bath. Formation of a pungent odor indicative of acrolein formation was recorded using a Portable Breath Checker. Due to the qualitative nature of this test, results were reported descriptively.

Detection of Hemoglobin-Like Compounds (Cyanmethemoglobin Method)

Preparation of Control Filters Containing Hemoglobin. Chicken blood was injected into a standard filter in amounts of 0, 100, 200, 300, 400, and 500 µL. The filter was then placed in an oven at a temperature of 50˚C for 12 hours. After drying, the filter was cut into thin pieces 0.5 cm long, dissolved in 5 mL of distilled water, incubated for 3 hours, and centrifuged at 3000 rpm for 15 minutes. 100 µL of the solution was transferred into a test tube, and 5 mL of Drabkin's reagent was added. The tubes were covered with aluminum foil, vortexed, and incubated at room temperature for 5 minutes. The absorbance was then measured using a spectrophotometer at 540 nm.

Measurement of hemoglobin levels in samples. The cigarette filter was cut into thin pieces 0.5 cm long, dissolved in 5 mL of distilled water, incubated for 3 hours, and centrifuged at 3000 rpm for 15 minutes. 100 µL of the sample solution was put into a test tube, and 5 mL of Drabkin's reagent was added. Next, the tube was covered with aluminum foil, shaken, and incubated at room temperature for 5 minutes, after which the absorbance was measured using a spectrophotometer at 540 nm. Results were expressed as mg hemoglobin equivalent per gram of filter. This analysis was intended as a preliminary screening and does not confirm the species origin.

Results and Discussion

Cigarette Filters

Most cigarette filters are made from non-biodegradable materials (de Granda-Orive et al., 2016) (Figure 1). However, filters have been understood to act as a barrier to remove toxins, thereby reducing damage. However, current evidence indicates that these filters do not provide significant health benefits for smokers because harmful cigarette smoke is still inhaled. This effect is also influenced by the filter fiber (National Center for Chronic Disease Prevention and Health Promotion (US) Office on Smoking and Health, 2014). Studies have shown that cellulose acetate fibers from cigarette filters can be inhaled, and microplastic fibers have been detected in human lung tissue, indicating that such particles may

accumulate in the respiratory system of exposed individuals (Mohammadi et al., 2025; Jenner et al., 2022).

Therefore, modifications to cigarette design may contribute to changes in the prevalence of histological types of lung cancer, with adenocarcinoma now being the most frequent. Gradually, the percentage of filtered cigarettes increased over time until it reached 97.5% in 1992. In the last 70 years, many materials have been patented for cigarette filters, but the most commonly used ones are paper, charcoal, and cellulose acetate (Hoffmann, 1997).

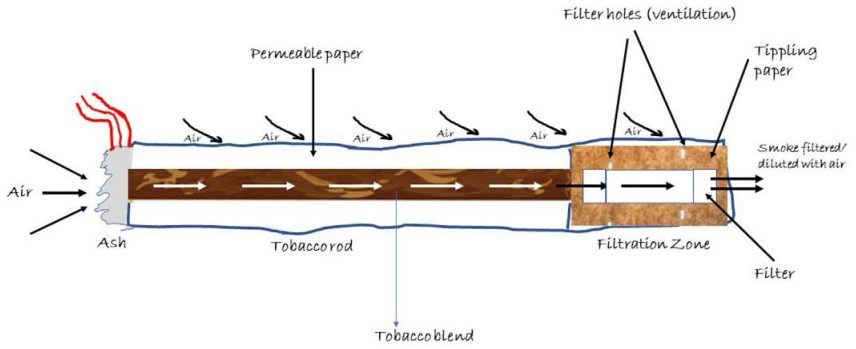


Figure 1. Schematic representation of a cigarette (de Granda-Orive et al., 2016)

The filter contains small ventilation holes for additional air that enters during inhalation. This filter functions to dilute the concentration of smoke and nicotine during smoking. Thus, smokers often prefer cigarettes with more ventilation holes because they taste lighter and smoother. This type of cigarette is also considered less dangerous (Carroll et al., 2021; King et al., 2021). In 1952, filter-tipped cigarettes accounted for only 1.4% of sales but increased to more than 40% of the market by the end of the 1950s (Federal Trade Commission, 2023). It has also been found that there is a causal relationship between tobacco use and cancer. In the 1950s, cellulose acetate filters began with claims that they could reduce the negative impact of unfiltered cigarettes on health (Everaert et al., 2023).

From the 14,123 participants, 7,056 (50%) were active smokers, 13,038 (92%) were white, and had an average smoking history of 60 smokers per year. Data showed that most individuals (12,488 [88%]) smoked filtered cigarettes. The results showed that unfiltered smokers had approximately 40% (hazard ratio, 1.37; 95% CI, 1.10-1.17) chances of developing lung cancer, and were almost twice as likely (hazard ratio, 1.96; 95% CI, 1.46-2.64) were more likely to develop lung cancer. Die from lung cancer compared to those who smoke filter cigarettes (Tanner et al., 2019).

The analysis results of Poorolajal et al. (2020) show that the addition of a 1 mm length of cigarette filter increases the absorption of benzene, arsenic, cadmium, chromium, and formaldehyde by 1.5%, 1.3%, 1.9%, and 2.0%, and 0.7%, respectively. In addition, the length of the cigarette filter significantly affects the absorption of harmful chemical components in cigarettes (Figure 2).

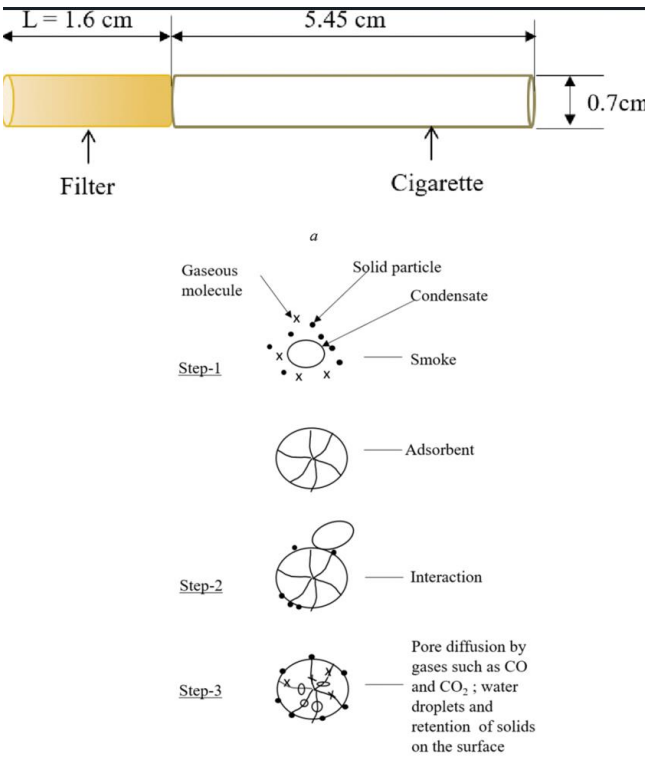


Figure 2. Cigarette filter working process (Kollati & Mohapatra, 2021)

Protein-Like Compounds in Commercial Cigarette Filters

The present study detected measurable protein-like compound concentrations in several commercial cigarette filter samples, ranging from non-detectable levels to 4.57 mg/g of filter (Table 2). Considering that cigarette filters are predominantly composed of cellulose acetate—a semi-synthetic polymer derived from plant cellulose—the detection of protein-like substances indicates the possible presence of additional materials beyond the primary polymer matrix.

Table 1. Protein standard cigarette filters

Protein (%)	Content of Protein (µg protein/g filter)
0	0
0.01	13.10
0.1	18.52
1	72.82
10	214.69

Cellulose acetate itself does not intrinsically contain protein structures (Wei et al., 2020). Therefore, the observed Bradford reactivity may be attributed to several possibilities: (1) surface-bound additives introduced during manufacturing, (2) residual tobacco-derived compounds migrating into the filter, (3) functional adsorbent modifications, and (4) non-specific interaction or matrix interference in

the Bradford assay

Table 2. The results of the analysis of protein samples cigarette filters

Coded	Form of Filters	Hydrophobic/ hydrophilic	Content of Protein (mg protein/g filter)
L1	White Fibers	Hydrophobic	1.56
L2	White Fibers	Hydrophobic	0
L3	White Fibers	Hydrophobic	1.13
L4	White Fibers	Hydrophobic	2.41
L5	White Fibers	Hydrophobic	1.32
L6	White Fibers	Hydrophilic	1.36
I1	Activated Charcoal	Hydrophobic	1.97
I2	White Fibers	Hydrophobic	1.21
I3	White Fibers	Hydrophobic	0
I4	White Fibers	Hydrophobic	1.78
I5	White Fibers	Hydrophilic	1.36
I6	White Fibers	Hydrophilic	1.22
I7	Activated Charcoal	Hydrophobic	1.90
I8	Activated Charcoal	Hydrophobic	4.57

It is important to note that the Bradford assay relies on the binding of Coomassie Brilliant Blue dye to basic and aromatic amino acid residues, and therefore does not confirm protein identity or biological origin. In addition, various compounds, including phenolic substances, aromatic compounds, and other colored extractives, may interfere with the assay and produce false-positive or elevated absorbance signals (Vallejo-García et al., 2026; Benjamin, 2023). Industrial literature indicates that cigarette filters have undergone multiple technological modifications, including the incorporation of activated carbon, flavor-release capsules, and adsorption-enhancing materials (Lim et al., 2023; Zhang et al., 2020).

Although certain patents have described the theoretical use of porphyrin-based complexes for gas adsorption (Stavridis & Deliconstantinos, 1994), there is currently no strong peer-reviewed evidence confirming the widespread commercial use of animal-derived protein in cigarette filters.

Therefore, the protein concentrations reported in this study should be interpreted as indicators of protein-like reactivity rather than definitive proof of intact protein or animal-derived substances.

Interpretation of Heme-Reactive (Hemoglobin-Screening) Findings

Heme-reactive values were detected using the cyanmethemoglobin (Drabkin) method in several samples. This assay is traditionally used to quantify hemoglobin through conversion to cyanmethemoglobin and spectrophotometric detection at 540 nm.

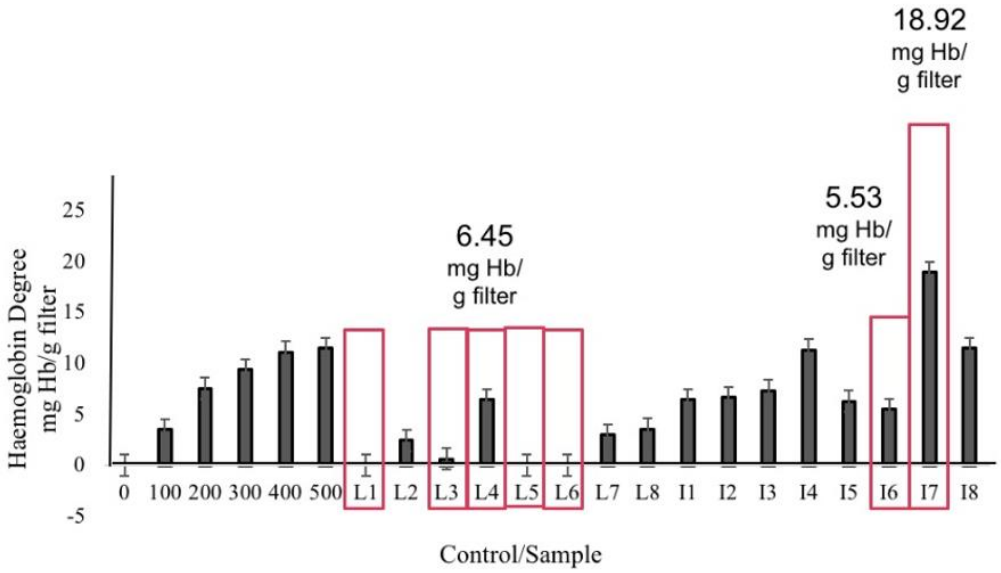


Figure 3. Hemoglobin Degree in Local and Imported Cigarettes

Hemoglobin (Hb) analysis results showed that samples L1, L3, L4, L5, L6, I6 and I7 showed Hb levels of 6.45 respectively; 5.53 and 18.92 mg Hb/g filter (Figure 3). The presence of Hemoglobin (Hb) has also been reported in the research results of Robiah (2012), who identified Hb in several cigarette filters using FTIR (Fourier Transform Infra Red). Pork and bovine hemoglobin, with filters without hemoglobin, appear in the second derivative of the infrared spectrum at wavenumbers 580-592 cm^{-1}. The spectral pattern of the second derivative of bovine hemoglobin has one peak and one valley, while pig hemoglobin shows one peak and two valleys. The second derivative results were validated using Lagrange interpolation, showing that the spectral pattern was valid. The types of molecular vibrations in the typical spectral region of pig and bovine hemoglobin at wavenumbers 580-592 cm^{-1} are asymmetric Fe-ring oblique bending vibrations (600-500 cm^{-1}), N-H bending (695-550 cm^{-1}), and C-C stretching (850-500 cm^{-1}), each of which has a different protein matrix environment

However, several analytical limitations must be considered: (a) the method detects heme-containing chromophores but does not confirm intact hemoglobin protein, (b) it cannot differentiate species origin (human, bovine, porcine, etc.), and (c) iron-porphyrin complexes or colored aromatic substances may produce spectral interference.

Cellulose acetate filters are typically plasticized using triacetin (glycerol triacetate) to bind the fibers and maintain structural integrity, while more advanced filter designs may incorporate adsorption-enhancing materials such as activated carbon to improve filtration performance (De Cesaris et al., 2024). Porphyrin-like structures, if present, could theoretically exhibit absorbance behavior similar to heme derivatives.

A patent document also describes the use of an additional component in the form of a metal–porphyrin complex (Figure 4). Structurally similar compounds are known to occur in hemoglobin, chlorophyll, and vitamin B12. Chlorophyllin has been reported to be very effective because it is relatively soluble in triacetin, allowing it to be applied during the manufacture of filters in adequate quantities through plasticizer dosage (Teufel et al., 2000).

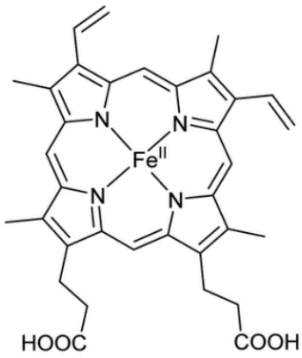


Figure 4. Porphyrin Structure in Heme (Haemoglobin) (Tahoun et al., 2021)

One of the cigarette filter inventions states that the porphyrin ring can retain harmful compounds contained in cigarette smoke (e.g., NO, NOx, carcinogenic nitroso compounds, free radicals, H_2O_2, CO, aldehydes, and trace elements), which until now have not been adequately retained by conventional cigarette filters (Figure 5). This refers to the general convention filter enrichment with biological substances of metal ions (Fe^{2+}, Cu^{2+}, Mg^{2+}) complexed with porphyrin rings and Fe^{2+} ions bound stereospecifically to protein molecules, either separately or in combination. Enrichment of these conventional filters with the biological substances mentioned above does not change the physical properties of cigarette smoke (smell, taste, and appearance) or the physical properties of the filter itself (Stavridis & Deliconstantinos, 1994).

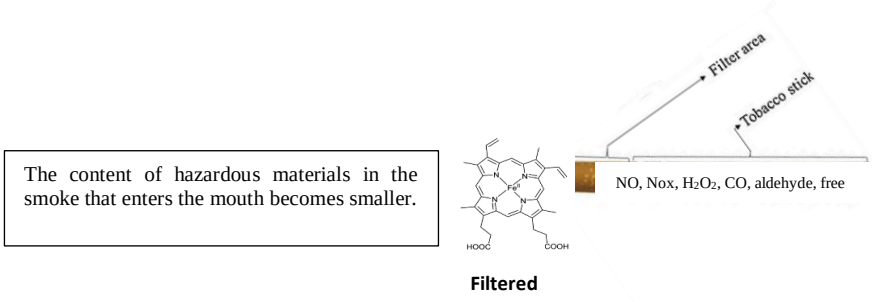


Figure 5. Hypothesis for the use of porphyrin compounds in cigarette filters

The amount of carbon monoxide released from the gas stream through a filter containing hemoglobin may increase significantly if the hemoglobin is accompanied by other factors found in the blood. The amount of carbon monoxide that can be released from the gas stream by hemoglobin depends on the equivalent

weight of the hemoglobin. However, in the presence of additional factors, the amount of carbon monoxide removed is much more significant than with hemoglobin stoichiometry alone (Scheinberg, 1976).

In another patent document, it is also found that there is an available cigarette filter characterized by a nanometer mesoporous material of immobilized hemoglobin self-assembled from an amphiphilic diblock copolymer of polyethylene glycol-polycaprolactone added to the drawing material, where the nanometer mesoporous material accounts for 10-25% of the total weight of the pulling material. The method of manufacturing a cigarette filter provided by the present invention comprises the following steps: (1) immobilizing hemoglobin in an amphiphilic diblock copolymer of polyethylene glycol-polycaprolactone with an average molecular weight of 8000, 6000, or 4000; and (2) adding HINH material (nanometer mesoporous material) to the cigarette filter pulling material. The cigarette filter provided by the present invention is intended to selectively reduce the biological hazard associated with CO in cigarette smoke. The immobilization conditions for PEG-PCL (polyethylene glycol-polycaprolactone) immobilized hemoglobin are mild, hemoglobin activity is guaranteed, the storage period is long, and the aroma of flue-cured cigarettes is not damaged (Na et al., 2011).

Given these limitations, it is scientifically more accurate to describe the findings as "heme-reactive compounds" rather than confirmed hemoglobin. Definitive identification would require advanced confirmatory analysis, such as LC–MS/MS proteomic profiling, Species-specific ELISA, Peptide mapping, and DNA-based authentication. These approaches would provide more accurate conclusions and minimize speculative interpretation.

Detection of Glycerol-Related Compounds

Qualitative acrolein testing indicated the presence of glycerol-related compounds in most filter samples. This observation is consistent with established industrial practices. An acrolein test was carried out to detect the presence of triglyceride molecules (Figure 6). Glycerol in free form or as part of lipid molecules will degrade when heated. This process results in the formation of the aldehyde molecule acrylate or acrolein, and fetid. Test compound acrolein using $KHSO_4$ as additional materials, forming acrolein (Sari et al., 2017). The principle of this test is based on the formation of acrolein, which is generally produced through the dehydration of glycerol, either in its free form or derived from fats and oils, via well-established catalytic reaction pathways (Abdullah et al., 2022; Ahmad et al., 2021; Babaei et al., 2020; Ma et al., 2020).

The acrolein test is based on the dehydration of glycerol, either in its free form or derived from fats and oils, under acidic conditions. In this process, potassium hydrogen sulfate ($KHSO_4$) acts as a dehydrating agent that promotes the conversion of glycerol into unsaturated aldehydes, primarily acrolein. Heating accelerates the reaction, and the formation of acrolein is typically indicated by a characteristic

pungent odor and the evolution of visible fumes (Ruoppolo et al., 2020; Li et al., 2020b; Babaei et al., 2020).

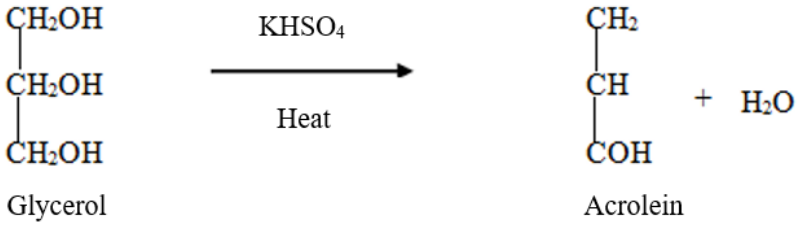


Figure 6. Formation of Acrolein

Triacetin (glycerol triacetate) is widely used as a plasticizer to bind cellulose acetate fibers and maintain the structural integrity of the filter rod (Zheng et al., 2020).

In addition, glycerol and propylene glycol are widely used as humectants in tobacco products, where they increase moisture retention and influence smoke characteristics, including aerosol formation and smoothness (Lin et al., 2020; Xizheng et al., 2018; Tong et al., 2021; Kassem et al., 2024).

Table 3. Qualitative analysis of glycerol in the sample of filter cigarettes

Coded	Intensity Measurements	Glycerol
L1	2	+
L2	1	-
L3	2	+
L4	2	+
L5	1	-
L6	4	+
I1	4	+
I2	2	+
I3	2	+
I4	4	+
I5	3	+
I6	3	+
I7	3	+
I8	4	+

(+) detected, (-) undetected

Glycerin has been identified as a natural component of oriental tobacco (0.34–0.48%), flue-cured tobaccos (0.07–0.12%), and burley (0.23–0.31%) (Rodgman, 2002). It is used as a processing aid during smoking as a humectant to prevent the tobacco leaves from becoming friable and thus crumbling during processing. It may also be applied directly to the cured tobacco during cigarette manufacturing as a flavoring material or vehicle. In the cigarette, glycerin helps to retain moisture and prevents drying out of the tobacco. Glycerin is typically applied

to tobacco at levels of 1–5% (Xizheng et al., 2018; Tong et al., 2021).

During the blending and processing of tobacco, humectants such as glycerin may be added to increase the tobacco's moisture-holding capacity and aid in processing. The cellulose acetate fibers are thin, white in color, and tightly packed to form a filter. These fibers are bonded with a plasticizer, triacetin (glycerol triacetate), which softens and welds them together into a cross-linked structure to provide firmness to the filter (Lin et al., 2020; Zhang et al., 2020).

The qualitative analysis showed that cigarette filter samples contained glycerol with different intensities, specifically in the samples L2 and L5 (Table 3). According to Bao-shan et al. (2020), increasing glycerol triacetate causes tar emissions to decrease gradually, total particulate CO and nicotine emissions are unchanged, and smoke water content increases slowly. This indicates that increasing the application of glycerol triacetate may be appropriate and could contribute to improved sensory comfort.

The results of these glycerol control measurements were used to standardize the intensity produced by the Portable Breath Checker. This means that when the scale shows one, then Glycerol is otherwise undetectable, and beyond is considered to be detected (Table 4).

Table 4. The measurement results in the glycerol control

Control	Intensity Measurements	Glycerol
Negative Control	1	-
Gliserol 100 μL	4	+
Gliserol 200 μL	4	+
Gliserol 300 μL	4	+
Gliserol 400 μL	4	+
Gliserol 500 μL	4	+

(+) detected, (-) undetected

In the tobacco industry, glycerin and propylene glycol can effectively maintain the moisture of tobacco leaves, improve the softness of tobacco leaves, and play an essential role in atomizing and carrying the smoke released from the thermal cracking of tobacco (Li et al., 2020a; Tong et al., 2021).

Humectants such as glycerol, propylene glycol, and trimethylene glycol have been added to tobacco products for many years to facilitate the processing of cured tobacco leaves, retain moisture, and increase shelf life (1–5). Humectants are hygroscopic substances that can maintain humidity (Chen et al., 2022).

Humectant concentrations vary significantly between different types of tobacco products (cigarettes, hookahs, etc.). For example, humectants in products such as cigarettes and pipe tobacco are added at levels that maintain moisture content without reducing the burning characteristics of the tobacco (Tong et al., 2021). According to Xizheng et al. (2018), glycerol and 1,2-propylene glycol are the humectants most commonly used by the tobacco industry. They are found in

various tobacco products and often at relatively high concentrations (~2–5 % w/w).

The acrolein reaction detects dehydration products of glycerol under acidic conditions; however, this test has several limitations: it is qualitative rather than quantitative, it does not distinguish free glycerol from triacetin and it cannot determine biological or synthetic origin. Therefore, while the detection aligns with expected formulation components, the method does not provide sufficient specificity for compositional profiling.

However, several studies have reported analytical investigations using advanced instrumentation to characterize tobacco raw materials, such as cigarette filters. An analysis employing pyrolysis–gas chromatography/mass spectrometry (Py-GC/MS) conducted by Ding et al. (2022) examined the chemical composition of cigarette filters. The results indicated that cellulose acetate is the dominant component of cigarette filters. The detected pyrolysis products included acetic acid, acetylated sugar derivatives, levoglucosan, and other fragments originating from the thermal decomposition of cellulose acetate. In addition to the primary polymeric material, the study also identified the presence of plasticizer compounds, particularly triacetin (glycerol triacetate)

Other studies have also reported the determination of glycerol, propylene glycol, and nicotine in refill liquids using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) operating in multiple reaction monitoring (MRM) mode with electrospray ionization (ESI). Kubica (2023) demonstrated that this analytical procedure is rapid, simple, and suitable for the simultaneous determination of these three analytes.

Instrumental confirmation using GC–MS or HPLC would enable: (1) quantitative measurement of glycerol and triacetin, (2) structural confirmation via fragmentation patterns, and (3) potential differentiation of production pathways through isotopic analysis. Thus, these approaches are proposed as important follow-up analyses for future studies.

Variability Between Brands

The data did not demonstrate a consistent pattern distinguishing local from imported brands in terms of protein-like or glycerol detection (Table 2 and Table 3). This suggests that filter additive strategies are influenced more by product design and manufacturer formulation than by geographic origin.

Activated carbon-containing filters exhibited slightly higher absorbance variability in some cases. This may be explained by: (1) Adsorptive retention of environmental compounds, (2) Co-extraction of colored materials, and (3) Matrix interference affecting colorimetric readings.

Halal Critical Point Assessment

From a halal science perspective, the presence of proteinaceous or glycerol-related compounds introduces a potential critical control point depending on their source. Halal risk does not arise from the chemical identity alone but from: (1)

Biological origin, (2) Slaughtering method (for animal-derived materials), (3) Cross-contamination during processing, and (4) Fermentation media (for biotechnological glycerol production).

Meanwhile, glycerol may originate from: (1) Petrochemical synthesis (generally halal), (2) Microbial fermentation (conditional halal), and (3) Hydrolysis of animal fat (requires source verification). Similarly, protein-based additives may originate from: (1) Plant sources, (2) Microbial production, and (3) Animal blood derivatives. Without source disclosure from manufacturers, risk assessment remains incomplete.

However, it is critical to clarify that this study does not demonstrate the presence of porcine hemoglobin. It only indicates reactivity consistent with protein-like and heme-reactive compounds.

Thus, from a halal regulatory standpoint, the findings suggest the need for transparency and traceability rather than immediate classification as non-halal.

Conclusion

The analytical results indicate that cigarette filters are not composed solely of cellulose acetate fibers, but also contain additional compounds such as glycerol-derived substances that function as plasticizers or humectants. Furthermore, preliminary screening revealed indications of proteinaceous compounds in several samples, although the analytical methods employed in this study were not able to conclusively determine their biological origin.

These findings suggest that cigarette filter components may represent a potential halal critical point if such materials originate from non-halal animal sources or from sources with unknown provenance. Therefore, further investigations employing more specific analytical techniques—such as proteomic analysis, biomarker-based LC–MS/MS, or DNA-based methods—are required to verify the origin of these substances. In addition, transparency in raw material sourcing and manufacturing processes is essential to support halal assurance systems, particularly for non-food consumer products that have rarely been examined within the framework of halal science.

Overall, this study provides an initial contribution to the development of analytical approaches for identifying halal critical points in tobacco-related products, while also opening opportunities for further research on material authentication and halal implications in non-food consumer goods.

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