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Chemical probe as specific detector of porcine protein or peptide in meat and meat-based products: Potential applications, challenges, and the way forward

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A B S T R A C T

This review discusses the potential use of chemical probes for detecting porcine protein/peptide in meat and meat-based products. Porcine protein/peptide detection is important for religious, cultural, and food safety reasons. Chemical probe has emerged as a potential tool for the sensitive and specific detection of various proteins. This review provides an overview of different types of chemical probes, their advantages, and limitations. The current and potential applications of chemical probes in the food industry are also discussed, along with the challenges that must be addressed for their effective use. The article concludes by emphasizing the importance of chemical probes as a valuable tool for porcine protein/peptide detection and the need for further research and development to advance their use.

1. Halal meat industry: a religion perspective

According to the State of the Global Islamic Economy Report 2022, the halal food market is the world's biggest consumer sector with the fastest growth of businesses worldwide. This is in line with data reported by Dinar Standard stated that the halal food market is experiencing substantial growth, projecting a market value of $1.4 billion and an anticipated growth rate of 6.9 %. Halal food is defined as food permissible for Muslims to consume and strictly prepared in accordance with Islamic dietary law, which forbids certain types of animals and follows the Islamic method of animal slaughter [1].

The Pew Research Center's Forum on Religion and Public Life reported that the Muslim demographic population is predicted to escalate to 35 %, from 1.6 billion in 2010 to 2.2 billion by 2030, as the second largest religion after Christianity. The growing Muslim population worldwide significantly indicates the rising demand of halal food. In 2021, Muslims spent $1.27 trillion on halal food, and this amount is expected to reach $1.67 trillion by 2025.

Contrastingly, Christian viewpoints may diverge from a strict adherence to the specific halal dietary mandates delineated in Islamic traditions. As a largest religious group worldwide with approximately 2.3 billion people, Christian consumers are the biggest prospects considering that faith-based marketing in halal food market [2]. This religion shared many common dietary and food system principles with Islamic practises. The increasing demand from Christian's customers is driving the growth of the halal market in Europe. Studies also showed some non-Muslim customers willing to pay extra prices for halal foods and thought halal food was of better hygiene and quality compared to non-halal foods [3]. This aligns with the agreement reported by Ref. [4] that Christian's consumers in Philippines was choosing halal food due to its quality, health and hygiene advantages.

Nonetheless, some Christians devotees may perceive eating halal foods as an assertion of personal choices that does not inherently conflict with their religious convictions. Moreover, others sides of Christians believers that consuming halal foods as part of their diet and reflecting for others religious practices and displaying a respecting and notable tolerant attitude [5].

Meanwhile, in Jewish perspective on halal foods is derived from the principles of kosher dietary laws. According to Jewish dietary laws, known as kashrut, certain foods are considered kosher (fit or proper) for consumption while others are not. The concept of kosher is similar to halal in that both involve specific rules and restrictions in food preparation and consumption. The kosher dietary laws of Jews were adapted from sacred texts, namely Torah [6,7]. The kosher dietary laws including animal slaughter, ingredient source, food preparation, and the separation of meat and dairy products. The strict nature of kosher dietary laws makes kosher meat also considered halal [8]. It is significant to note that the Jewish community has various opinions on the permissibility of consuming halal foods (Table 1).

Meat and meat products are among the most popular halal foods that

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Table 1
Comparison of dietary law among abrahamic faiths.

CHARACTERISTIC	ISLAM (Halal)	Christianity	Judaism (Kosher)
Prohibition of Pork	Forbidden	Varies by denomination	Forbidden [49]
Ethical Slaughter	Zabihah Method [50]	No explicit rules	Shechita Method [51]
Prohibition of Blood	Obligatory to drained	No explicit rules	Blood must be drained
Alcohol Beverage	Forbidden	Varies by denomination	Alcohol permitted; wine must be kosher [52]
Seafood	All fish considered halal	Varies by denomination	Must have fins and scales to be kosher [53]
Meat & dairy product	Meat from permitted animal & must ethical slaughter	Varies by denomination	Strict separation required [54]

highest in demand [9–11]. Halal meat are the one of the highest demands in commodity market. Recent research has suggested that by 2023, the expected growth in consumption of various meats such as poultry, pig meat, beef, and sheep meat will be 15 %, 11 %, 10 % and 15 %, respectively. Poultry meat is anticipated to 41 % of total meat protein intake in 2023, followed by pig, bovine, and ovine meats. Apart from the United States, Brazil, and China, the large increase in meat consumption is forecast to be more significant in low-income countries, notably India, Pakistan, the Philippines, Vietnam, and the Sub-Saharan region of Africa. Over the past decade, global per capita meat consumption has fluctuated in the range of 6 kg per capita, and this trend is expected to continue throughout the predicted timeframe [12].

Kabir [13] stated that demand for halal meat from Muslim consumers is increasing due to the increasing number of Muslim populations worldwide. Moreover, awareness and exposure toward the halal concept among non-Muslims also increase their experiences with halal food because of its cleanliness and safety. In addition, meat and meat products play an essential role as a source of proteins, enriched with vitamin B and mineral elements such as ferum and selenium in dietary components [14].

Although there is an increasing interest in plant-based meat analogs as a dietary trend to substitute animal meat [15], the majority of consumers still choose animal protein due to their higher quality protein, which is important for maintenance, supports muscle protein synthesis, and is necessary for the repair of damaged tissues [16]. Animal protein provides higher quality protein because it produces higher amounts of essential amino acids (EAA) in adequate quantities than plant protein [17].

However, due to the great demand, meat trade capitalists eye the opportunity to make profit by fraudulent practices such as substitution and mixing of good quality meat with another cheaper meat [18]. Therefore, meat fraud by mislabelling, fake descriptive label information, and fraudulent product marketing have become widespread in consumers society and making it difficult for legal enforcement to arrest the perpetrator meat cartel [19,20].

2. Meat and meat-based products adulteration issues

Food fraud scandal and adulteration occurred in the food industry as early as the 1800s when corrupted food businesses replaced bread and beer with chalk and sugar, respectively [21]. Food adulteration is the process of lowering or reducing the quality of food by replacing of food ingredients with unauthenticated substances or by removing a crucial component from food for making money and profit [22].

There are various types of food adulteration from milk and fruits to vegetables and grains. Nevertheless, the scope of the current review will focus on meat and meat-based products adulteration. Although some meat adulteration was not a significant major problem because the meat can be disguised by processing to a limited amount, due to religious beliefs, it is a crucial issue in the global Muslim community. As a result, this sparks an alarming reflection for the Muslim community in their cautious attitude toward adulteration of meat and as a driving cause of halal meat demand. Meat and meat-based products are among the food products of interest that face adulteration and fraud, including substitution by pork derivatives, usage of blood plasma, addition of prohibited substances, usage of pork intestine casings, and fake halal meat [23,24].

The major concern among the community pertaining to meat and meat-based product adulteration is inter-species substitution. The act of incorporating pork meat in the manufacturing of meat and meat products has been deliberately carried out by irresponsible producers to gain high margin profit. Pork meats are often becoming the best candidate for fraudulent use because beef and pork meat are likely quite similar, cheaper, and difficult to distinguish, except using certain markers and authentication techniques [25]. The nearly similar colour, appearance and taste of the pork meat with beef, mutton or other poultry is nearly impossible to distinguish and identify with naked eyes. Moreover, pork was the most prevalent undeclared species in burgers, sausages, and frozen meat [26]. This agrees with cases involving meat products such as hamburgers, patties, meatballs, sausages, and salami that are frequently vulnerable to adulteration with pork meat [27].

In Indonesia, several cases have been reported involving adulteration of pork in meatballs [28–32]. Meanwhile, few cases in Egypt of meat samples such as burgers, kofta, luncheons, and sausages were positively adulterated with porcine meat [26,33]. The same scenario was observed in South Africa [34] and Iran [35] where, pork was the most common animal species for substitution and adulteration in meat products. Interestingly, although pork is the most popular meat in China, beef meat is still adulterated with pork meat to gain the highest profit because beef is expensive in China [18,36].

3. Detection technique for the authentication of adulteration

A decade ago, the most common popular authentication method for food adulteration relied on the physicochemical and electrophoresis methods. In line with the development of research and technology, numerous authentication procedures have been developed to address the demand for more efficient and rapid detection methods. Nowadays, most commercial porcine detection kits use biological-based detection approaches such as DNA. Polymerase chain reaction (PCR) is among the DNA-based techniques that commonly used to identify the presence of specific materials in meat products due to their high specificity and sensitivity [37–39]. Its specificity has become a favorite method for the detection of pig DNA in meat products and to examine whether porcine substances exist in food, especially meat and meat products [40] (Fig. 1).

However, these approaches are expensive, time consuming, require suitable storage conditions, and laborious [41]. Garibyan and Avashia

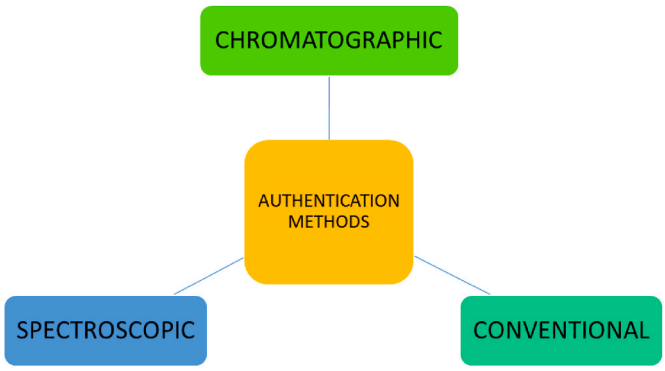

Fig. 1. Authentication methods.

[42] reported that PCR is inclined to errors by increasing the risk of false positives in the generated fragments. In addition, PCR products are also prone to be changed by non-specific primer binding to other similar sequences and unpredicted amplicons from the primer dimer [43].

Furthermore, there are other detection approaches using chemical-based porcine probes that have been developed to diversify the method of authentication. Nevertheless, this method mostly uses probes labeled with fluorescence dyes such as SYBR green [44], chemiluminescent [45,46], and radioactive isotopes [47], which require special labeling techniques as well as expert technicians to perform the procedures.

Currently, there are three different methods of halal authentication that have been developed to identify meat adulteration (Table 2). The race in developing sophisticated methods of halal authentication was developed from different states of samples, which range from crude protein to the molecular level [48].

4. Limitations of current authentication methods

Despite the diversity and sophisticated of the tools for halal authentication, each of the methods still has limitations either in terms of sample preparation, condition parameters, laborious procedures, and cost or the need of expertise in handling the methods. Thus, in line with the growing for meat demand and the availability of numerous meat products in the market from unknown sources, a rapid halal screening technique that is easy, rapid, and economical is necessary. From 1980 to 2022, there has been no clear trend in the methodologies used for halal verification [92]. Before the polymerase chain reaction (PCR) trend in 1984, researchers commonly used conventional methods, such as dielectric and electrophoresis for halal authentication. Since its inception for halal authentication, PCR has been widely used for electrophoresis and has become a popular method in the laboratory. Over the decades, the research and development of authentication technology has driven researchers to actively find the best method for improving existing halal authentication techniques.

However, in their quest to refine the existing methods, some limitations and drawbacks in some techniques were identified. Ng et al. [92] reported that high performance liquid chromatography (HPLC) instruments are expensive and require expert personnel to handle. Prior to running the test, the right chromophore/fluorophore is needed for derivatization because amino acids are unable to absorb radiation. In addition, this technique sometimes damages the samples, and requires a laborious extraction method, and takes a long time for a single analysis [56].

Meanwhile, in techniques that require DNA and protein as a sample, such as PCR, extra care and caution is needed because of the sample characteristics. PCR is excellent in providing a dependable, rapid, sensitive, and highly specific [93] technique for species identification, but this technique is tedious regarding to sensitive sample preparation [94]. The PCR technique requires the selection of a suitable DNA/RNA extraction method and sufficient DNA template for analysis. DNA template is also prone to degradation even by mild heating. Each technique has several limitations as some processed meat samples must undergo thermal treatment. The effect of thermal treatment with high temperature and pressure includes defects in protein and DNA structures, which are necessary for the induction of analysis and may reduce the credibility of identification in meat adulteration. Interference in protein structure may result in changes in the conformational epitope for target protein binding of antibody sites, which can lead to improper binding [95,96]. Meanwhile, the instability of the DNA structure might affect the amplification process because fewer fragments can be amplified, which can increase the potential of non-specific detection [97].

Despite the efficiency of ELISA for meat adulteration identification [73], the composition of meat products, which vary in fat content and processing techniques, limits detection from product to product. In addition, owing to its intricate nature, optimal performance and efficiency may not be achieved before implementation. This might be because of sample preparation, protein and lipid extractions, and instrument use [98]. With regards to the effectiveness of the electrophoresis method, PCR and ELISA assays can discriminate the meat species in meat adulteration. Nevertheless, these approaches are time consuming [99,100] and susceptible to cross-contamination [101–103].

On the other hand, the requirement of proteolytic enzymes in most proteomics and genomic procedures, such as the use of trypsin, may possibly lead to the destruction of samples, which can alter the results [104,105]. This led to a work by Samodova et al. [106] that reported ProAlanase as an effective alternative to trypsin because of improvements in protein sequence coverage. In addition, the insufficiency of data available as reference among researchers for interlaboratory comparison is another limiting factor in food authentication [107]. The lack of a standard protocol can be a bias factor during the interpretation of data analysis. This is because the factors influencing an experiment can vary and if not recorded and maintained, can lead to unreproducible results [108]. The lack of reliable analytical methods [109] for future studies and validation of the effectiveness of discriminators can diminish the effort required to introduce powerful authentication tools. Hence, a database specifically for food authentication is a must concerning sampling methods, including sample unit, sample variability, sample size, and sample storage [110,111].

All of these issues have become significant springboards that have sparked the idea of solving the current problems; by designing and developing more practical, cost-effective, and rapid methods in halal authentication. Therefore, a new approach that is capable of producing significant results and can serve as important guidelines is needed to improve the existing halal authentication system.

Table 2
Methods for halal authentication of meat and meat products.

METHODS	TECHNIQUES	REFERENCES
CHROMATOGRPHIC	High-Pressure Liquid Chromatography (HPLC)	[55–59]
	Gas Chromatography (GC)	[60,61]
MOLECULAR	Capillary-Electrophoresis (CE)	[62–64]
	Electric Nose (EN)	[65–67]
	Polymerase Chain Reaction (PCR)	[68–71]
	Enzyme-linked ImmunoSorbent Assay (ELISA)	[72–74]
	Differential scanning calorimetry (DSC)	[75]
SPECTROSCOPIC	Nuclear Magnetic Resonance	[76]
	Fourier Transform Infrared Spectroscopy (FTIR)	[77–80]
	Fluorescence Light Spectroscopy	[81]
	Near-Infrared Spectroscopy (NIR)	[82–85]
	Laser Breakdown Spectroscopy (LIBS)	[86,87]
	Raman Spectroscopy (RS)	[88–90]
	Dielectric Spectroscopy	[91]

5. Chemical probe: an alternative

Hence, along with the development of advanced technology, various halal authentication techniques have been developed to identify the authenticity and adulteration of meat and meat-based products. At present, authentication of halal status in meat and meat products is critical. Halal meat is a subject matter that is particularly important to certain customers with religious beliefs. Therefore, the need for a reliable and effective halal meat detection tool has become a big challenge [92], where the use of chemical probes as a detection tool is an alternative in detecting halal meat fraud.

Chemical probes play an important part in understanding the role of proteins or peptides by assisting in function analysis. The term chemical probe is often used interchangeably. Nonetheless, the term chemical probe that has frequently been used by researchers is a specific small molecule that acts as regulator of a protein's activity that enables studies

of the molecular targets mechanistic and phenotypic characteristics in biochemical, cell-based, diagnostics, or animal experiments [112,113]. It is simply a compound reagent [114] with specific probe characteristic that allow them to precisely and efficiently bind to certain biomolecular targets, such as nucleic acids and proteins, allowing researchers to examine their roles and interactions in biological systems [115]. This powerful tool is extensively used in drug discovery, chemical biology, and authentication development to decipher complex mechanistic processes and identify potential therapeutic targets [113].

Development of chemical probes for protein/peptide binding requires a critical understanding of the structure and function of the target biomolecule and computational methods such as molecular docking to anticipate the interactions between the probe and the target [116,117]. Moreover, to ensure the effectiveness of chemical probes as protein/peptide detectors, several critical variables such as specificity, sensitivity, and stability must be meticulously studied [114].

Generally, the scientific processes of designing chemical probes for food applications are nearly similar with the design of probes for other various applications. But the uniqueness of a chemical probe is varying in terms of their methodological approaches, probe criteria, binding targets in which they interact with, and the functional output upon the probe's binding. Intriguingly, in the field of chemical probe design, recent studies by Ref. [118] discusses the designing of chemical probes employing *dual steric* approach. The authors provided several step-by-step guides starting from determination of target sites that is suitable for probe binding. It is then followed by generation of pharmacophores which have high binding potency and selectivity to the sites of interest. The steps continue with the design of molecules that can incorporate with the pharmacophores to ensure their binding with the target sites. Then, the binding affinity and specificity of the probes are evaluated to test for potency and selectivity. Subsequently, the chemical probes are validated through an array of biochemical and biophysical assays such as fluorescence-based or spectroscopy-based assays for detection of sensitivity and specificity. Next, optimization phase to refine the chemical probes and to sharpen their performance and minimizing false-positive results were carried out. Lastly, verification of chemical probe is the final stage to determine the safety, efficacy and reliability of the chemical probes with other authentication systems that are available in the market [118].

In the context of essential criteria that a chemical probe should be meet, studies by Ref. [115] introduced '*fitness factors*' term when selecting and assessing chemical probes. The fit-for-purpose guidelines refer to the desirable criteria of a chemical probe that should be considered to determine its suitability and effectiveness. These criteria encompass various aspects such as chemical properties, biological potency, biological selectivity and context of use. Table 3 shows a set of guidelines criteria of chemical probe for researchers to consider when choosing the appropriate chemical probe.

These factors have a significant impact on the ability of chemical probes to accurately detect their target biomolecules [119]. The probe must exhibit sensitivity that binds only to the chosen target biomolecule and extreme specificity in detecting even trace amounts of the target [120]. Additionally, the probe must be robust enough to maintain its structural integrity throughout the detection process to provide reliable and trustworthy results.

Intriguingly, in line with the advancement of technology and research in chemical probes, a web portal has been developed to facilitate researchers in selecting high-quality chemical probes for their researches. Inaugurated in 2015, the public resources, named Probes Portal (https://www.chemicalprobes.org/) was established as an online repository by an expert authority in the fields of chemical biology and drug discovery. Chemical Probes Portal became expert-led assessment portal of chemical probes that offering valuable advice on probe selection and use, as well as expert recommendations on probes. It is a free online resource that helps researchers to select and use high-quality chemical probes for their needs. This peer-reviewed portal provides critical evaluations of small-molecule probe used to study protein function and biological processes (Fig. 2).

Table 3
Guidelines criteria for selection of chemical probe.

Criteria	Threshold Value
Aqueous solubility	>0.05 mg/ml in low % DMSO aqueous solutions
Membrane permeability	Permeability essential; minimal PGP-mediated efflux in cell lines of interest
Chemically reactive groups	None present unless a well characterized and selective mechanistic requirement
Molecular weight (Da)	Likely to be <450
Lipophilicity (LogP)	Likely to be <5
H-bond donors (*O*–H, *N*–H)	Likely to be <3
H-bond acceptors (N, O)	Likely to be <11
Rotatable bonds	Likely to be <10
Target potency (IC50 or Ki)	10_7–10_9 M
Target selectivity	Well-defined selectivity; >10–100-fold against closely related targets; polypharmacology undesirable
Mechanism of action	Well-defined quantitative relationship between biochemical and cellular effects consistent with target-dependent action
Pharmacokinetics	Good pharmacokinetics not essential for in vitro and cellular use, but required for in vivo animal work

To date this non-profit portal contains more than 500 compounds with 400 proteins target and approximately 100 protein families. Through this portal, complete information from 1069 expert reviews from 214 panel of academic and industry experts on the right use of chemical probes is provided to improve the reliability as well as to facilitate the discovery and development of new pharmaceuticals [113].

6. Chemical probe application

Over the years, the field of chemical probes has quickly changed, with a growing emphasis on the creation of innovative probes capable of detecting specific targets with great sensitivity and selectivity. Peptide-based probes have attracted substantial interest recently among various other types of chemical probes due to their potential utility in targeted detection and imaging of various biomarkers in both diagnosis and treatment [121,122].

For example, Wang and Hu [123] developed a peptide-based probe for tumor biomarker identification. These probes can detect and photograph particular biomolecules in aqueous medium and live cells, providing a novel tool for biomarker identification and monitoring.

In recent years, the creation of antibody-based probes has made it possible to identify chemical substances such as fenpropathrin in samples of fruits and vegetables in real time and to dynamically monitor cell surface proteins [124,125]. These probes have several possible uses, including testing for food safety and medical diagnoses.

The potential of activity-based probes to characterize enzymes throughout the full proteome has led to their increasing popularity. By observing the activity and localization of deubiquitination enzymes in live cells, Fang et al. [126] studied activity-based probes to examine the function of these enzymes in cellular processes. This study reveals how activity-based probes can help us understand how enzymes work.

Numerous studies have been conducted on fluorescent probes, especially with their potential use in determining and monitoring cell viability [127]. The quantity and localization of reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulfur species (RSS) in living environments have also been monitored using these sensors. Fluorescent probes have been shown by Ref. [128] to be capable of identifying many species in a single biological sample. Therefore, there are several applications of fluorescent probes in biology and medicine.

Affinity probes have also demonstrated potential in the separation and analysis of phosphopeptides, which can help in the understanding

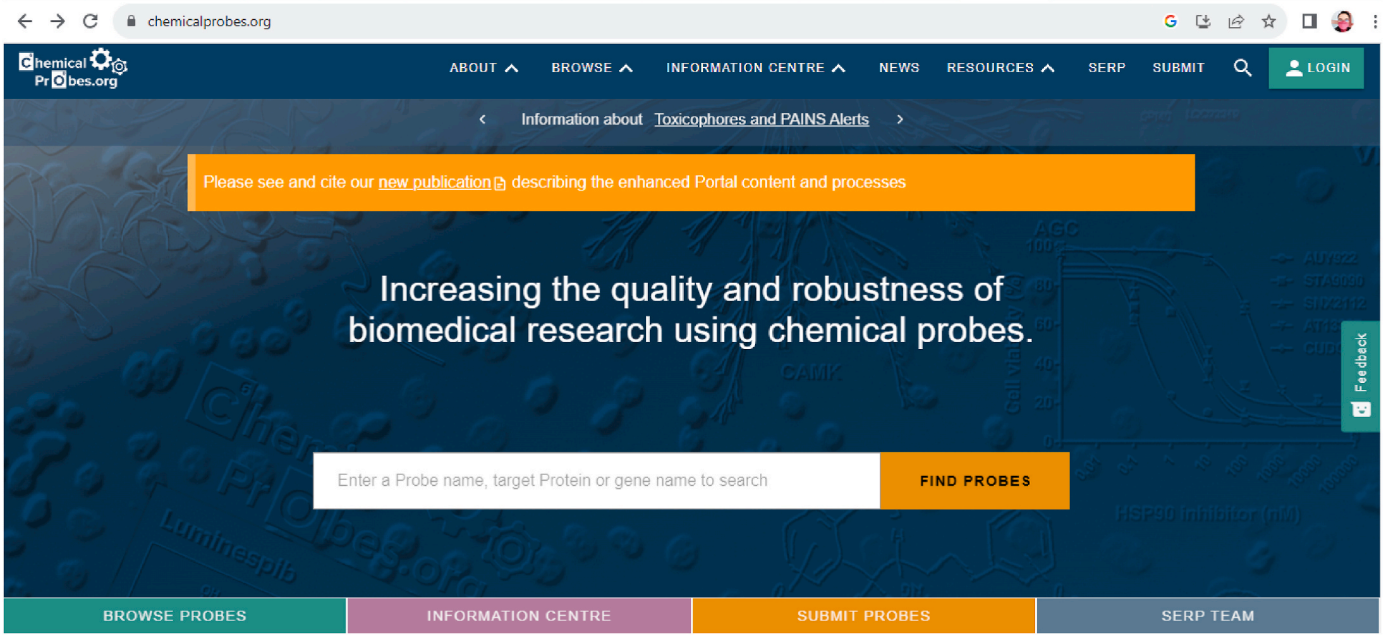

Fig. 2. Interface of chemical probe portal.

many biological processes [129]. Photoaffinity probes are helpful tools for researching lipid-protein interactions. Thus, the use of chemical probes has important implications for various disciplines, including environmental science, biotechnology, and medicine. By enabling the identification and monitoring of specific targets with extreme sensitivity and selectivity, these probes provide a cutting-edge method for research and diagnostics. Table 4 shows the types of chemical probes with their target detection and applications.

On top of that, chemical probes play a crucial role in food quality assurance to ensuring the safety and integrity of food systems. Moreover, it also functions to detect and quantify food adulterants, pathogens, or contaminants with high sensitivity and selectivity. By integrating chemical probes into routine monitoring processes, food industry professionals can rapidly identify potential food adulteration, assess the freshness and authenticity of products, and enforce regulatory standards. This approach not only protects public health but also enhances consumer confidence in the food supply chain, fostering a culture of healthy, hygienic, and excellence in food production and distribution.

The report from Ref. [18] highlighted the potential of spectroscopy-based sensors in detecting fraudulent minced meat substitution. A total 120 samples of beef with bovine offal and pork with chicken were subjected to visible and fluorescence spectra and multispectral image acquisition. The MSI-based models scores varying from 87 % to 100 % while Vis-based model scores varying 57 %–97 %. The study by Ref. [133] proposed an optimized electronic nose system (OENS) as an authentication method for detecting pork adulteration in beef. The study analyzed seven classes of meat, comprising different mixtures of beef and pork. The findings showed an accuracy of 98.10 % using the optimized support vector machine. This study can plays an important role of food authentication for ensuring halal compliance. Next, a new oligonucleotide-based electrochemical biosensor was developed by Ref. [134] to detect the *Sus scrofa* mitochondrial cytochrome *b* (cytb) gene. This study utilized a screen-printed carbon electrode (SPCE) modified with graphene (Gr) and gold nanoparticles (AuNPs) composite as a detection platform. The proposed detection has the potential to be applied for pork meat adulteration that beneficial for food safety and quality control.

In addition, study by Ref. [135] reported the use of a perylene probe induced by a cationic polymer to identify changes when milk was adulterated. While, findings from Ref. [136] which developed a Lossy mode resonance (LMR) based fiber optic sensor for the detection of adulteration in milk. Nearly similar study reported by Ref. [137] that used titanium dioxide (TiO_2) as both an indicator and a probe that can

Table 4
Types of chemical probe.

Types of Chemical Probe	Detection Targets	Applications	References
Peptide-based probe	Tumor biomarkers	Development and potential use of novel peptide-based molecular probes for the targeted detection and imaging of different biomarkers in both diagnosis and therapy	[123]
	Biomolecules in aqueous media	Detection/monitoring of biomolecules in aqueous media and in live cells.	[130]
Antibody-based probe	Protein	Real-time imaging of the cell surface protein	[124]
	Organic compound	Detection of fenpropathrin in vegetable and fruit samples	[125]
Activity-based probe	Enzymes	Proteome-wide enzyme profiling	[126]
	Enzymes	Study the activity and localization of deubiquitination in living cells	[131]
Fluorescent probe	Cell	Detect and monitoring cell viability	[127]
	Reactive oxygen species (ROS), Reactive nitrogen species (RNS), and Reactive (redox-active) sulfur species (RSS	- Monitoring the concentrations and locations of the species - Detect the presence of more than one species in the biological environment	[128]
Affinity probe	Peptide	Isolation and study of phosphopeptides	[129]
Photoaffinity probe	lipid	Study of lipid-protein interactions	[132]

quantitatively identify adulterants in milk. All these studies are important findings in the development of probe-based detection methods for adulterated milk in the food industry for ensuring the safety and authenticity of dairy products.

7. Utilizing computational strategies for the design of chemical probes

The use of porcine-specific proteins or peptides as templates to design specific chemical probes for detecting porcine in meat-based products is expected to offer several advantages. Firstly, choosing peptides are highly specific to porcine proteins that are absent in other animal species may ensure a precise detection of porcine protein/peptide in meat mixtures without producing false positive results. Secondly, they may exhibit high sensitivity, enabling the detection of even trace amounts of porcine protein/peptide in meat and meat-based products, which is crucial for accurate detection [138].

Contemporarily, computational methods or *in silico* methods are gaining popularity and offer effective techniques in a multitude of chemico-biological applications as well as in the pharmaceutical industry [139]. The implementation of computational methods in molecular design and drug discovery is part of modern experimental theoretical and computational techniques [116]. These methods are useful for reducing the use of animal testing [140], assisting in the design of novel probes and safe drugs [141], and aiding research scientists throughout drug discovery processes [142].

In this context, *in silico* studies are being applied to accelerate and search hit identification [143–145], hit-to-lead optimization [146,147], design chemical probes, molecular profiling and pharmaceutical toxicity [148,149] as well as food safety industries [150–152]. A number of newly invented drugs and probes have been designed through *in silico* procedures as a promising strategy for the identification of novel drug or probe entities [153].

Generally, two *in silico* methods are commonly used in drug design, in which the application can also be extended to design or screen potential chemical probes, namely ligand-based and structure-based methods. In the ligand-based method, only the information of known ligands that bind specifically to a target site is available. This method can be used to predict the relationship between the physicochemical properties and bioactivities (commonly known as structure-activity relationship) of potential ligands or probes [153]. Second, structure-based approaches using methods such as subject-target docking (proteins or RNA), which are based on the identification of binding sites and interactions for their respective functions. This method requires the information about the target structure [154].

For example, molecular docking simulations can be used to evaluate the binding specificity and affinity of potential probes to specific porcine proteins/peptides. Based on their 3D structures, molecular docking predicts the binding mechanism and affinity of a ligand such as a chemical compound to a receptor; such as a porcine protein/peptide [155]. The chemical compound was screened to have probe properties comparable to those of the porcine protein/peptide binding site, allowing it to attach to the target molecule.

Thus, the use of *in silico* computational methods is proposed to be beneficial in improving the design and screening of chemical probes which will allow assessment of binding affinity, specificity, and stability of the porcine protein/peptide-chemical compound complex predictions. This approach offers a reduction in both time and cost involved in the analysis of porcine protein/peptide-chemical compound complex. Moreover, these computational techniques are not widely explored in probe design, rendering them as an attractive option to conventional experimental methods.

8. Challenges and the way forward for chemical probes as specific detectors

The development of chemical probes that are specific to porcine protein or peptide face great challenges to detect the porcine protein or peptides efficiently. A chemical probe that can discriminate between the target protein/peptide and other protein/peptides in the sample matrix remain as big hurdles to be solved in probe design. This is crucial because numerous proteins may have nearly similar peptide sequences, making distinction difficult. Furthermore, the probe must be safe to use and not impair the quality of the meat product.

It is critical to consider the sensitivity and selectivity of the chemical probe while detecting porcine protein/peptide in meat and meat products. As a result, a robust, reproducible, and low limit of detection and quantification technique is required to ensure precise and accurate results. Furthermore, it should be capable of identifying target proteins/peptides in a variety of sample matrices, including complex food matrixes. Ideally, the probe should be easy to use and provide a fast analysis time for high-throughput analysis. Additionally, it is important to consider the cost and availability of the probe to ensure its wide distribution for routine meat fraud detection.

Thorough validation is essential in the development of chemical probes that can accurately and specifically detect porcine protein/peptide in meat and meat-based products. The probe must undergo rigorous validation procedures, which may include testing in various matrices of meats and comparison with other authentication procedures available in the market and in laboratory settings in order to ensure reliable and trustworthy results.

9. Conclusion

In conclusion, detecting porcine protein/peptide in meat and meat-based products is critical for food safety and halal demand from Muslim customers worldwide. Chemical probes with high sensitivity and specificity to specific porcine protein/peptide offer alternative promising solution for this problem. On the other hand, developing chemical probes that are specific and sensitive enough to identify porcine protein/peptide is an arduous task. The likelihood of cross-reactivity with other protein/peptide, which can result in false positive or false negative findings, is a big challenge. Hence, it is critical to construct probes that can distinguish between structurally nearly identical protein/peptide and to execute reliable results upon testing via rigorous validation methods. Chemical probes hold the potential to become vital tools in verifying the safety and authenticity of meat products and which can meet the increasing demand for rapid, precise and reliable authentication procedures of meat fraudulent. This highlights the significant potential of chemical probes in halal meat industry.

CRediT authorship contribution statement

Mohd Nurhadi Hamsar: Writing – review & editing, Writing – original draft, Investigation. **Awis Qurni Sazili:** Supervision, Resources, Conceptualization. **Siti Farah Md Tohid:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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