

## Recent advanced techniques in cysteine determination: a review

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### Article history:

Received: 23 April 2020

Received in revised form: 31 May 2020

Accepted: 8 June 2020

Available Online: 3 August 2020

### Keywords:

Cysteine,  
E920,  
Food additives,  
Food security,  
Analytical method

### Abstract

The utilization of cysteine in a wide variety of products especially bakery products has led to a huge concern of various groups of consumers especially those who restricted to religious-based dietary. It has become a major concern due to the raw materials are derived from arguable sources such as pig bristles and human hair. This review briefly elaborates cysteine as food additives with highlighted issues in *halal* perspective and toxicity in the food industry. This review also highlighted several analytical approaches used in direct determination of cysteine compound such as high performance liquid chromatography (HPLC), molecular imprinted polymers (MIPs), Raman spectroscopy, flow injection spectrophotometric, electrochemical biosensor and gold nanoparticles based calorimetric assay

### DOI:

dfid

## 1. Introduction

Cysteine is an amino acid common to many proteins and enzyme. It is of interest due to the presence of a reactive thiol group as a side chain (Hunt, 1985; Gmunder *et al.*, 1990; Demirkol *et al.*, 2004). As a control functionalization of thiols, cysteine has it important roles in detoxification of heavy metals in living organisms, antioxidant capabilities of tissues and mitochondria, blood coagulation in mammals, transport across cell membranes and electrochemical sensing (Sirko *et al.*, 2004; Wirtz *et al.*, 2004; Wirtz and Droux, 2005; Borase *et al.*, 2015; Cebi *et al.*, 2017). Cysteine usually found in relatively low concentration in dietary protein which does not exceed 5% of total amino acids (Demirkol *et al.*, 2004; Ismail *et al.*, 2014). They can be found in whole foods such as meat, grains, nuts, fruits and vegetables. Production of cysteine in the worldwide market can reach up to 400 tons (Berehiou *et al.*, 2013) where the major production was used as food additives (Ismail *et al.*, 2014). It acts as a stabilizer in bakery ingredients by softening the texture of the yeast and preventing from further oxidation (Wada and Takagi, 2006; Wu, 2013; Ismail *et al.*, 2014) while in animal food production it is been used as artificial flavour by

mimicking meat flavour (Cao *et al.*, 2017).

Nonetheless, cysteine production raised an ethical issue and consumer concern upon the source of the raw materials. The availability of cysteine food additives in the food industry is majorly derived from keratin hydrolysis (Ryu *et al.*, 1997; Berehiou *et al.*, 2013; Ismail *et al.*, 2014). Precisely, the keratin sources are extraction product from human hair or animal parts such as feathers, bristles or hooves (Frape *et al.*, 1971; Cebi *et al.*, 2017). Cysteine extraction from human hair is widely used due to its abundantly available and low-cost production. Apart from that, cysteine production might come from the fermentation process. The process involved enzymatic bioconversion from two type bacteria strains which *Pseudomonas* sp. and *E. coli* (Sano and Mitsugi, 1978; Tamura *et al.*, 1998). The enzymatic bioconversion method has been used by Ajinomoto Co. Inc., Japan in industrial scale (Wada and Takagi, 2006; Ismail *et al.*, 2014). The doubtful sources of cysteine may be criticized in different aspects especially in religion practice consumers. The goal of this review is to cover recent advances in cysteine determination techniques. This review also discusses the physical and chemical structure of cysteine, its

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applications specifically in the food industry, and food safety issues of cysteine as additives.

## 2. Cysteine amino acids and its religious issue

Amino acids (AA) are important for growth and development. It acts as precursors for building up proteins and other biologically important substances such as peptides, hormones and enzyme (Baker, 2009; Kimura, 2014; Poinot *et al.*, 2016; Koderia *et al.*, 2017). It is categorized as essential AA that can be obtained from the diet and non-essential AA that is synthesized directly in the human body. Accordingly, cysteine is a non-essential AA as it can be directly synthesized in the body. However, the classification of essential AA should comprise the ability of the organism to synthesize carbon skeleton or just nitrogen compounds (Wu, 2009). Since cysteine carbon skeleton cannot be synthesized directly, thus it is under nutritionally essential AA (Wu, 2009).

Cysteine is a polar uncharged AA containing thiols – SH side chain (Demirkol *et al.*, 2004). It takes part in protein synthesis, cellular metabolism, stabilizer and also detoxification (Borase *et al.*, 2015; Cebi *et al.*, 2017) and important for stabilization of tertiary and quaternary protein conformation through disulfide bridges (Wirtz and Droux, 2005). In addition, protein associated and free thiols are responsible for the binding of metals and react with nucleophilic drugs and reactive oxygen (Plaza *et al.*, 2018; Yin, 2015). The conversion of free thiol groups to disulfide bridges and vice versa constitutes a dynamic reactive system that is the basis for redox switches in protein (Wirtz and Droux 2005).

According to the food additives database from European List, cysteine is labelled under the E numbers of E910, E920 and E921 which are L-Cysteine, L-Cysteine Hydrochloride and L-Cysteine Hydrochloride Monohydrate, respectively. It falls under the antioxidant category that the proportions of the added cysteine amount shall not be greater than the maximum permitted proportions according to Food Regulations 1985 (Ministry of Health Malaysia, 1985). According to Food Drugs and Administration (FDA) the maximum volume of cysteine addition in dough is up to 0.009 parts per 100 parts as well as in flour bakery products.

Keratin hydrolysis is a huge production for cysteine additive. The keratin sources can become from four different sources which are pig bristles, human hair, duck feather and cow horn and it is considered as the major process production of cysteine in China (Ismail *et al.*, 2014). The importance of cysteine in bakery ingredient required a cost-effective production to meet supply demand. Therefore, acid hydrolysis of keratin has been used by the manufacturers to produce cysteine (Ryu *et*

*al.*, 1997; Berehoiu *et al.*, 2013; Xu *et al.*, 2013). In order to obtain one kilogram of cysteine in keratin hydrolysis, at least 27 kg of HCl is needed and temperature must be set at 100°C for 6 hours (Berehoiu *et al.*, 2013). Hence, human hair is chosen as one of the raw materials due to abundantly available for mass production. But there are also animal sources like feathers, bristles or hooves to be used as cysteine sources.

Based on the primary source of AA extraction it would rise some circumstances related to religion perspective especially on Muslim community.

*“...Indeed, what He has forbidden to you is the flesh of dead animals and blood and the flesh of swine, and that which has been sacrificed to anyone other than Allah...” (2:172-173)*

It is clearly being mentioned in the Quranic verse above that God prohibited anything related to swine for humankind to consume. This is included anything from head to toe and the essence of the swine itself including anything that related to the animal. The prohibition of swine is not solely purposed to obey God’s rule but it is also related to some diseases in human (Nurdeng, 2009; Ali, 2014). This raised an ethical issue and consumer concern about its safety for consumption. It is offering some advantages which the human hair is cheap and it requires one ton of human hair to produce 100 kg of cysteine (Berehoiu *et al.*, 2013). But, extraction of cysteine from human hair may produce an unpleasant odour, required a large volume of HCl, costly and not environmentally friendly (Ismail *et al.*, 2014).

According to fatwa released by Department of Islamic Development Malaysia (JAKIM), any types of food that derived from the human body (hair) considered as haram (MS1500, 2009). Precisely, if cysteine’s source is extracted from pig bristles and human hair it clearly violates *halal* principles which mentioned that whatever conducive to the haram is itself *haram* (Al-Qaradawi, 2007). This is because cysteine extracted from pig bristles and human hair may lead to epidemic generated from those sources. Consequently, it will relate to another *halal* principle which mentioned the prohibition of things due to their impurity and harmfulness (Al-Qaradawi, 2007). In order to protect and maintain the integrity of *halal* status, cysteine derived from animal-based is urged to be banned from entering Malaysia and mandatory posed for correct labelling ingredients of the source content (Idris, 2016). Meanwhile, according to the Turkish Food Codex Regulation on food additives, cysteine (E920) used as a food additive is banned in wheat flour (Cebi *et al.*, 2017).

### 3. Analytical methods for cysteine determination

The presence of cysteine and related thiols compound in bakery products, animal feeds, pharmaceutical and cosmetic products have increased demand for a reliable method in order to determine the authentication of cysteine's raw material. For the past few years, the implementation of *halal* guidelines by Malaysian government showed that the consumers demand quality and safe end products. Thus, Department of Islamic Development Malaysia (JAKIM) has been taking an initiative by implementing *halal* guideline in Malaysian standard (MS1500:2009 *Halal* Food – Production, Preparation, Handling and Storage – General Guidelines) to ensure the quality and safety issue of end products. As been mentioned before, cysteine is used as food additives in bakery products and nutrient supplement in pharmaceutical and cosmetic products. Hence, the determination of cysteine should be taken into measured among the authorities for consumer safety and *halal* issue. To address this need, several methods have been developed and published for the determination

of cysteine (Table 1).

#### 3.1 High performance liquid chromatography (HPLC)

Recently, high performance liquid chromatography (HPLC) has been proven to be one of the commonly used instruments for the analysis of biological matrices and pharmaceutical preparations due to its high efficiency and good reproducibility. This method is able to determine concentrations of total cysteine content in wheat flour, bovine, porcine and fish gelatin (Azilawati *et al.*, 2015; Lamp *et al.*, 2018). Unfortunately, cysteine compound is often neglected in the amino acid analysis (AAA) through HPLC analytical measurement (Dai *et al.*, 2014). This is because cysteine is susceptible to oxidation and reduction during hydrolysis mechanism (Barkholt and Jensen, 1989; Dai *et al.*, 2014). Consequently, cysteine is prone to incomplete recovery in most of HPLC's AAA. This can be explained when thiols group that contains sulfur side chain may lead to the formation cysteine sulfinic/sulfenic/sulfonic acid due to reaction with residual oxygen during acid hydrolysis (Lamp *et al.*, 2018). In a study done by Bartolomeo and

Table 1. Analytical methods for determination of cysteine

Method	Approach	Description	References
Chromatographic	High performance liquid chromatography	Identification of cysteine amino acid through amino acid analysis (AAA)	Bartolomeo and Maisano (2006) Dai <i>et al.</i> (2014) Azilawati <i>et al.</i> (2015) Lamp <i>et al.</i> (2018)
	Raman spectroscopy	Determination of cysteine sulfhydryl bond (S–H) stretching vibration is a unique probe of local sulfhydryl (SH) structure and dynamics at wavelength 400-450nm.	Li and Thomas (1991) Amir <i>et al.</i> (2013) Demir <i>et al.</i> (2015) Fu <i>et al.</i> (2018)
Spectroscopic	Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR)	Characteristics cysteine amino acids based on the polar functional group. The side chain of sulfhydryl (SH) and amino groups (NH <sub>2</sub> ) can be represented in amide I and amide II band.	Subramanian <i>et al.</i> (2009) Demir <i>et al.</i> (2015) Hell <i>et al.</i> (2016) Chávez-Murillo <i>et al.</i> (2018) Chen <i>et al.</i> (2018) de la Rosa-Millán <i>et al.</i> (2018)
	Flow injection spectrophotometric	Direct determination of cysteine through derivatization with 18-molybdophosphate (18-AMP) and o-phthalaldehyde-N-acetyl-cysteine (OPA-NAC).	Blasco <i>et al.</i> (1997) Giljanović <i>et al.</i> , (2011) Petrova <i>et al.</i> (2016)
Electrochemical biosensor	Carbon paste electrode	Electrochemical response is due to the reaction between designated carbon paste electrode and cysteine.	Santhiago and Vieira (2007) Brinic <i>et al.</i> (2013)
	Molecular imprinted polymer (MIP) and ultraviolet spectroscopy (UV-VIS)	A combination of designated specific polymer and UV-Vis for cysteine compound identification.	Cai <i>et al.</i> , (2014) Hashemi <i>et al.</i> (2017)
Collective	Gold nanoparticle and UV-VIS	Detection of cysteine compound is due to aggregation of cysteine compound around gold nanoparticles.	Shang <i>et al.</i> (2007) Schulz and Baranska (2007) Rastegarzadeh and Hashemi (2015)
	ATR-FTIR and Raman spectroscopy	Identification of cysteine side chain groups disulfide (S-S) and sulfhydryl (S-H) by utilizing both instruments capabilities.	
Alternative method	Fluorescence probe	Changes in phosphate buffer solution color intensity after addition of cysteine.	Wang <i>et al.</i> (2018)

Maisano (2006), tryptophan and cysteine amino acids are destroyed during acid hydrolysis while serine and threonine suffered from partially lost in protein biopharmaceutical analysis.

Meanwhile, a mixture reagent of peptide synthesizer contained trityl-resin, fluorenylmethyloxycarbonyl chloride (fmoc) glycine, S-trityl-Fmoc-L-Cysteine and Fmoc-glu-tert-butyl ester were used to hydrolyze cysteine and other protein in wheat flour showed a great detection limit, 0.72 nmol/g in HPLC analysis (Reinbold *et al.*, 2008). The detection is based upon the protein extraction from tens of different types of wheat flour. Based on cysteine detection in wheat flour, Reinbold *et al.* (2008) proved that a low concentration of glutathione and cysteine in flour from S-deficient wheat had a similar effect on the technological properties as the altered composition of gluten proteins.

### 3.2 Molecular imprinted polymers (MIPs)

Molecular imprinted polymers have been recognized due to their advantages involve in predetermined recognition ability, chemical stability, simple preparation and relative ease. Initially, this approach has received much attention in the fields of polymer science and chemical analysis. Theoretically, MIPs are synthetic polymers belong to specific cavities designed for a target molecule and are synthesized by the polymerization of different components (Lin *et al.*, 2012). The stability of MIPs is based on adsorption capacity, active site accessibility and fixed shape (Hashemi *et al.*, 2017). Hence, due to low-cost and easy preparation, MIP has gained wide acceptance as molecular recognition materials in developing sensor.

Hashemi *et al.* (2017) had used a combination of spectrophotometric detection (UV-Vis) based on MIP grafted with multiwall carbon nanotubes (MWCNTs) for separation and detection of cysteine in water samples. This method is based on the functionalization of MWCNTs by methacrylic acid (MAA) and subsequent polymerization for the preparation of a selective MIP/MWCNTs composite for separation and enrichment of cysteine prior to its spectrophotometric determination. Tremendously, MIPs able to identified cysteine with a LOD as low as 2.3ng mL<sup>-1</sup>. In fact, cysteine in human serum and water samples was successfully determined with recoveries ranging from 96.6% to 102.4%.

Similarly, Cai *et al.* (2014), performed fluorescence determination of cysteine by using MIPs as an adsorbent with newly synthesis fluorescent detector chemodosimeter of rhodamine B derivative (RB1). The precipitation polymerization of cysteine MIP was prepared by mixing MAA, ethyleneglycol dimethacrylate

(EGDMA), acetonitrile and methanol. A novel chemodosimeter, RB1 has LOD up to 12.5 nM to enhance the extraction of MIPs. This method successfully separated cysteine with other amino acids in aqueous solution.

### 3.3 Raman spectroscopy

Raman spectroscopy is a method to determine the chemical structures of molecules and interactions of molecules by representing the frequencies of molecular vibrations (Nemecek *et al.*, 2013). The molecular vibrational will represent the peptide backbone and its side chains in protein analysis. The spectral positions, intensities, and polarization of the Raman bands are sensitive to protein secondary, tertiary, and quaternary structures and to side-chain orientations and local environments. The Raman band resulting from the cysteine sulfhydryl bond (S-H) stretching vibration is a unique marker of sulfhydryl (SH) structure (Nemecek *et al.*, 2013).

The sulfhydryl will be used as Raman marker which occurs in a region of the spectrum between 2500 cm<sup>-1</sup> to 2600 cm<sup>-1</sup> (Nemecek *et al.*, 2013). The interaction between sulfhydryl hydrogen bonding and the S-H stretching frequency yield a distinct wavenumber value for cysteinyl SH group identification. Meanwhile, a study done by Fu *et al.* (2018) recorded the wavenumber of SH group of crystal cysteine occurred at 2552 cm<sup>-1</sup> and 2546 cm<sup>-1</sup> (SH stretching) and 1070 cm<sup>-1</sup>, 1000 cm<sup>-1</sup> and 944 cm<sup>-1</sup> (SH bonding). This difference of recorded wavenumber because of additional weak hydrogen bonds are by the SH group either with oxygen or sulfur atom (Minkov *et al.*, 2009).

A study done by Baranski and Baranska (2008), showed that cysteine compound in the tobacco and carrot plant can be detected by using Raman spectroscopy. The spectroscopy of cysteine compound was further validated with hierarchical cluster analysis (HCA) in order to differentiate between genetically modified (GM) crops and non-genetically modified (Non-GM) crop. In addition, the wavenumber of 500-600 cm<sup>-1</sup> was used in the experiment as a set of a cluster which based on the amide and S-S stretching band. Therefore, the presence of additional S-S bonds in the GM samples could explain the differences observed between the GM and non-GM spectra in a low wavenumber range. Thus, other than the sulfhydryl group used as a marker, peptide S-S group of cysteine can also provide helpful information for a reliable interpretation.

On the other hand, Cebi *et al.* (2017) used a combination of Raman microscopy with chemometric analysis of PCA and HCA, to differentiate ten types of

wheat flour into the adulterated group and the non-adulterated group. The analytical technique was able to detect cysteine compound with LOD of 0.125% (w/w). Raman spectral range was set between 500 – 2000  $\text{cm}^{-1}$  prior to performing PCA and HCA analysis. The perfect combination of Raman spectroscopy and chemometric analysis was successfully discriminated and classified between non-adulterated and adulterated wheat flour.

### 3.4 Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR)

Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy is an internal reflection light incidence from a denser medium to lower index medium (air or sample) with an incident angle greater than critical angle  $\theta_c$  (Vongsvivut *et al.*, 2014). The condition cause the electromagnetic field (evanescent wave) decays exponentially in limited depth into the adjacent lower index sample. Any infrared (IR) absorbing material locating in contact to the interface interacts with the evanescent field causing a reduced IR intensity. The reduction of IR intensity is measured reflect to incident IR radiation and transformed into an ATR-FTIR spectrum (Vongsvivut *et al.*, 2014). For example, polar functional groups (e.g. carbonyl compound) could easily be detected because of IR depends on the change of the permanent dipole moment of a chemical bond (Käppler *et al.*, 2016).

Determination of cysteine compound residue by ATR-FTIR is widely introduced in wheat flour. This is because cysteine residue is one of the subfractions in wheat flour (Li *et al.*, 2006). Many studies, found that valuable structural characteristics related to the interaction between protein components and amino acids sample that could be obtained from amide I and amide II band (Manley *et al.*, 2002; Reinbold *et al.*, 2008; Amir *et al.*, 2013; Hell *et al.*, 2016; Cebi *et al.*, 2017; Chagas *et al.*, 2018; Chen *et al.*, 2018). The detection relied on the organization of the chemical structure in a sample. The protein organization structure can be identified in ATR-FTIR through specific wavelength changes (de la Rosa-Millán *et al.*, 2018). For example, the difference of amide I band which is assigned around 1600 – 1700 $\text{cm}^{-1}$  can detect stretching vibration of C=O and C-N groups. Meanwhile, the amide II around is located around 510-1580  $\text{cm}^{-1}$  in terms of in-plane N-H bending (40-60%), C-N (18-40%) and C-C (10%) stretching (Amir *et al.*, 2013). Thus, it could give us valuable information on the possible interactions that occurred at the molecular level that produce a significant impact on cysteine identification.

In a study done by Li *et al.* (2006), glutenin subfraction which consists of cysteine residue had been

successfully differentiated between hydrated flour, gluten protein and flour. The result showed that hydrated flour had the highest peak in the amide I region at 1633  $\text{cm}^{-1}$  and 1612 $\text{cm}^{-1}$  (Li *et al.*, 2006). This can be supported by data from Chávez-Murillo *et al.* (2018) where different proportions amide I band ratio at 1625 and 1616  $\text{cm}^{-1}$  were recorded in different heat starch conditions. The study proved that heat treatment could rearrange the  $\beta$ -helix structure of protein in the flour. Moreover, ATR-FTIR analysis also proved that difference between  $\alpha$ -helix and  $\beta$ -sheet secondary structures of the amide II group in different flour extracts (de la Rosa-Millán *et al.*, 2018). Hence, it proved that, the capability of ATR-FTIR spectral measurement to determine the chemical composition of protein content qualitatively.

Apart from that, the major advantages of using ATR-FTIR are a rapid, nondestructive, time-saving method that can detect a range of functional groups and is sensitive to changes in molecular structure. ATR-FTIR could provide information on the basis of chemical composition and the physical state of the whole sample (Amir *et al.*, 2013). Thus, makes it a valuable tool in compound identification.

### 3.5 Flow injection spectrophotometric

Most research used chromatographic analysis for cysteine detection in AAA. However, cysteine is hard to be measured because it can oxidize easily due to the presence of thiols compound (Blasco, 1997). Flow injection spectrophotometric is introduced for cysteine detection based on rapid proceeding redox reactions or on the derivatization of cysteine. Flow injection spectrophotometry is advantageous with high reproducibility and throughput up to 180 samples per hour by photometric detection (Petrova *et al.*, 2016).

In order to improve rapidity of cysteine determination, Petrova *et al.* (2016) had used 18-molybdophosphate (18-AMP) as a derivative reagent and a fabricated modified chip of polymethyl methacrylate (PMMA) by flow injection spectrophotometric in cysteine dietary supplement. Accordingly, the maximum spectrum observed reaction between 18-AMP and cysteine was 820nm. This method able to detect aqueous solution of cysteine (from  $1 \times 10^{-5}$  to  $1 \times 10^{-4}$  M) with a LOD of 0.03mM and 240 samples can be determined per hour. Meanwhile, Blasco *et al.* (1997) had used o-phthalaldehyde-N-acetyl-cysteine (OPA-NAC) as a derivative reagent to detect cysteine in pharmaceutical capsule sample. Cysteine analytical range was  $1.0 \times 10^{-5}$  to  $1.0 \times 10^{-4}$  M. Yet, this method is proved to determine large many samples at a time with good reproducibility performance. Giljanović *et al.* (2001) had determined

cysteine without sample pretreatment by using flow injection spectrophotometric method. The determination of cysteine is based on the reaction of Pd<sup>2+</sup> ions with cysteine to form yellow Pd<sup>2+</sup>, cysteine complexes with the LOD 5.84 μmol/l.

### 3.6 Electrochemical biosensor

Apart from fluorescence detection of cysteine by HPLC, electrochemical biosensor has also been applied to identify cysteine. This biosensor with an electrochemical transducer and is considered to be chemically modified electrode (CME) as electronic conducting, semiconducting or ionic conducting material. Additionally, electrochemical sensor is advantageous over other methods because of their low cost, fast response and low limit detection (LOD) (Zhou *et al.*, 2007; Wang, *et al.*, 2015). Theoretically, electrochemical response is due to the reaction between specific electrode designs with cysteine compound (Dharmapandian *et al.*, 2010). Hence, lead to specific signal cysteine determination. Electrode accumulation is employed to provide concentration required for analysis by flameless atomic absorption spectrometry and anodic stripping voltammetry. In this sense, electrochemical methods present advantages of simplicity with easy miniaturization and high sensitivity (Brinić *et al.*, 2003).

Brinic *et al.* (2013) developed bismuth film random array carbon fiber microelectrodes (BiF-RACFMEs), in conjugation with square wave cathodic stripping voltammetry (SWCSV) to determine cysteine in the dietary supplement. A carbon fiber electrode is applied due to their advantages over other carbon-based materials, such as decreasing of capacitive current, increasing of mass transfer rate, faster equilibrium time and negligibly ohmic drop. Cyclic voltammograms obtained at BiF-RACFMEs in acetate buffer (pH 4.5) correspond to the deposition and dissolution of cysteine concentration. Hence, reduction current of bismuth cysteinate, formed on BiF-RACFMEs at pH 4.5, is used as analytical signal for determination of cysteine and NAC. The detection limit is 0.028 μmol/l, in the case of cysteine concentration between 1 μmol<sup>-1</sup>L and 10 μmol<sup>-1</sup>L with sensitivity up to 398 nAμmol<sup>-1</sup>L.

In a study done by Santhiago and Vieira (2007) modified carbon paste electrode was successfully determined cysteine in pharmaceutical formulations. The electrode was modified with the introduction of laccase of the fungi *Aspergillus oryzae*. Fungi enzyme (laccase) was used in this study due to fast response to a specific substrate and highly selective. The determination of cysteine compound was based on the inhibition effect of cysteine compound with metal ions Ag<sup>+</sup>, Zn<sup>2+</sup>, Hg<sup>2+</sup>. The result showed that the recovery rate of cysteine is 87%

and above and lifetime of the biosensor can be up to 9months. Meanwhile, cysteine desulhydrase enzyme (EC.4.4.1.1) was incorporated to determine cysteine compound in the pharmaceutical product (Hassan *et al.*, 2007). The rate of cysteine by-products (sulfide ion) was potentiometric ally quantified as cysteine concentration. The result showed the concentration range of cysteine compound between 0.2–150 mgL<sup>-1</sup> with a lower detection limit as low as 1 μmolL<sup>-1</sup>.

### 3.7 Gold nanoparticles based colourimetric assay

The interaction of gold nanoparticles (AuNPs) with cysteine and its derivatives is the basis of a number of bio nanotechnologies, and for these, the most important process is aggregation (or ant aggregation), which enables an array of colourimetric detection methods (Acres *et al.*, 2014). The mechanism of aggregation will be observed when AuNPs were functionalized with cysteine and its dimer cystine, or the cysteine-derived tripeptide. The aggregation is can be seen when cysteine forms a two-layer boundary around the gold nanoparticles. The cysteine induced aggregation of AuNPs without further pH modification: the first by interparticle zwitterionic interaction and the second by interparticle hydrogen bonding (Abraham *et al.*, 2010).

In order to improve the detection of cysteine using gold nanoparticles, Shang *et al.* (2007) had modified the method by prepared fluorescent conjugated polymer-stabilized gold. The addition of cysteine, a thiol-containing amino acid, the fluorescence of the colloidal solution increases significantly, indicating that cysteine can modulate the energy transfer between fluorophore and gold. This phenomenon then allows for sensitive detection of cysteine with a limit of detection (LOD) of 25 nM. The linear range of determination of cysteine is from 5 x 10<sup>-8</sup> to 4 x 10<sup>-6</sup> M. Another study showed that cysteine compound was successfully detected in tablet capsule with a limit of detection to 2.7 μmmolL<sup>-1</sup> through the combination of gold nanoparticles and UV-Vis spectrophotometer (Rastegarzadeh and Hashemi, 2015). Hence, a precise yet sensitive gold nanoparticle probe is a good analytical measure for cysteine compound detection.

### 3.8 Application of chemometric analysis

Chemometric is a combination of statistical and mathematical methods into chemistry. In particular, chemometric is unsupervised multivariate methods allow for more advanced treatment of data derived from chemical mixtures into simplest forms. It provides a large number of tools for pattern recognition, classification and identification which could increase the speed and meet the analysis objective (Christensen and

Tomasi, 2007). For example, transmittance values from ATR-FTIR and Raman spectroscopy could be used as a variable to determine intercorrelations between measured variables (Oliveri and Simonetti, 2016). The exploration of measured variables from various analysis tools discussed above is advantageous in order to transform data based on origin sources. There are several tools applied in the chemometric analysis which are principal component analysis (PCA), hierarchical analysis (HCA), partial least squares regression (PLS) and orthogonal projections to latent structures discriminant analysis (OPLS-DA). Combination of PCA, HCA and Raman spectroscopy were successfully differentiating GM crop and non-GM crop of carrot and tobacco plants (Baranski and Baranska, 2008). The wavenumber range between 500-600  $\text{cm}^{-1}$  was selected throughout this study as a variable marker. This can be supported by a study done by Cebi *et al.* (2017) where a combination of HCA, PCA and Raman microscopy differentiate ten types of wheat flour into the adulterated group and the non-adulterated group. On the other hand, ATR-FTIR were able to discriminate a total of 144 honey floral sources according to their geographical origin with the aid of HCA and PCA (Gok *et al.*, 2015). The samples were grouping based on spectral range 1800-750  $\text{cm}^{-1}$  in HCA. In addition, ATR-FTIR spectroscopy successfully differentiated bread wheat species with the combination of PCA and HCA (Demir *et al.*, 2015). The whole ATR-FTIR spectra were used in order to cluster the samples into a separated group.

#### 4. Conclusion

Cysteine has a good prospect in the food industry to be recognized worldwide. As a part of the protein, cysteine is an essential nutrient additive that may benefit for the consumers. However, the production of cysteine remained scarce for consumer trust. There is a need for method development in the determination of cysteine. Spectroscopic methods such as Raman and ATR-FTIR is a versatile analysis technique for determining the chemical composition of cysteine compound. This is due to the ability of light ray to absorb thiol linkage of cysteine compound. The analysis is robust and required minimal sample preparation. In addition, the combination of chemometric analysis such as PCA or HCA could increase the efficiency and reliability of the result.

#### Acknowledgement

This work was supported by the IIUM Research Initiative Grant Scheme [RIGS16-068-0232] from the International Islamic University Malaysia (IIUM), Selangor, Malaysia.

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