Fluorescence Determination of Acrylamide in Potato Chips Based on P540 and P503 Fluorescent Reagents

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ABSTRACT: *Acrylamide is one of the potential environmental public health problems, resulting from its increased accumulation in the process of cooking foods containing high levels of carbohydrates that are fried or cooked at high temperatures. So, developing a precise and sensitive analytical method for detecting and determining acrylamide in foods is absolutely necessary and inevitable. Gas Chromatography (GC) and Liquid Chromatography (LC) are two main laboratory techniques for acrylamide determination. In this study, we tried to use a cheaper, faster, accurately method for measuring acrylamide in real food samples. P540 and P503 were used as fluorescent reagents to detect the concentration of acrylamide in potato chips samples from 4 companies. Fluorescence spectroscopy was used in this work. The results obtained for the detection of acrylamide in comparison with the HPLC-MS method showed that there is an acceptable overlap between the fluorescence spectroscopy and HPLC method. The amount of acrylamide in four potato chips samples, obtained from the market in Tehran city, was determined using the proposed method. The optimum values of different parameters were determined. Comparisons between two methods, HPLC-MS and fluorescence spectroscopy were also described. The figures of merit for the proposed method were in the ideal range. The developed methods showed a high correlation coefficient (R^2= 0.991). According to the results of the fluorescence emission spectroscopy and its comparison with HPLC-MS, the performance and reliability of the proposed method as a simple, efficient, and rapid method with reduction of cost and time for determining acrylamide in potato chip samples were demonstrated.*

KEYWORDS: *Potato chips, Acrylamide; Fluorescence Spectroscopy; HPLC-MS method; P540 and P503 fluorescent reagents.*

INTRODUCTION

Acrylamide (2-propenamide) is an industrial chemical material that is used to produce poly acrylamide and its copolymers. These compounds are used for refinement of tile industries and paper wastewater, purification of

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drinking water, cosmetic industry, and ore processing. Acrylamide is characterized by its carcinogenicity, toxicity to the nervous system, and toxicity to genes. In April 2002, the Swedish National Food Authority (NFA) and researchers of Stockholm University published their findings on the high levels of acrylamide in high-fired or cooked foods which is rich in carbohydrates. This report has caused concern around the world. It was founded that acrylamide is formed during the thermal process of starchy foods [1]. Therefore, it is absolutely necessary and inevitable to develop a precise and sensitive analytical method for detecting and determining acrylamide in food. Some research about the process of acrylamide formation and its reduction in the past decade has shown several key factors that may affect the formation of acrylamide in food. In general, the amount of available precursors (asparagine, reduced sugars) is clearly decisive for the reaction, and in most cases, asparagine should be reduced in food [2]. Some factors such as product type, storage conditions, and seasonal weather conditions affect asparagine level and consequently affect the formation of acrylamide in fried potatoes during cooking. Another approach has shown that the material processing conditions of food (baking temperature, time, and moisture content) are also effective. In general, the higher acrylamide will produce in the food by increasing the cooking temperature. The European Food Safety Authority (EFSA) confirmed the previous assessment of acrylamide in foods based on the studies in 2014, which potentially increases the risk of cancer for consumers in all age groups. They state that acrylamide is a neurotoxin in animals and humans and is a multi-species carcinogen in both male and female rodents [3]. Food rich in carbohydrates such as cereal and potato products has higher acrylamide compared with foods rich in protein such as meat and fish. The production of acrylamide occurs mainly from the browning of the mail lard reaction and in the high temperatures so that the factors such as pH, moisture, nutrient reagent, and temperature affect its formation. Reducing sugars and asparagine amino acids are the basis of acrylamide formation [4]. Acrylamide is an unsaturated and hydrophilic compound with a high boiling point and can be connected to a double bond in other unsaturated compounds through its double bond and create a polymer. This compound is rapidly absorbed into the skin, and when it is corrosion, quickly absorbed and distributed to the tissues [5]. Also, as an electrophilic compound, it can react with nucleophiles in an increasing reaction, and thus attacks an enzyme and protein structure such as sulfide in reduced glutathione and then reacts [6]. Acrylamide is converted into oxidative epoxy glycidamide by reacting with the enzyme involved in the cytochrome and can connect to hemoglobin and disrupt its activity. Acrylamide has become a more active compound in the body, called glycidamide, and it is a mutagenic compound that promotes the cancer process potato chips are the main source of its. Although, acrylamide is present in processed cereals such as biscuits, crackers, bread, cakes, and cookies, as well as coffee and cacao. The acrylamide mutation activity in humans and mice is due to the ability of glycidamide to react with DNA. In addition, both acrylamide and glycidamide can react fast with blood hemoglobin and enzymes [7]. This material is a by-product of a variety of baking processes such as baking, frying, grilling, and roasting with temperatures up to 120 degrees centigrade in high carbohydrate products. The conversion of the asparagine amino acid to acrylamide in potatoes occurs in the presence of sugars, while the formation of acrylamide of carnosine in red meat does not require sugar. The high acrylamide levels are identified in fried potato products, such as fried potatoes and potato chips [8]. In the potato frying process, the formation of acrylamide is start at a temperature above 120 °C and reaches its maximum at about 170-180 °C, depending on the heating time [9]. Some reports indicate that the level of acrylamide in potato slices after frying for about 7 minutes at 150 °C is about 500 μg/kg, which after frying for 3.5 minutes at 190 °C is about 4500 μg/kg [10]. Studies have shown that high levels of reducing sugars are more effective than high levels of asparagines in the primary substance of acrylamide formation. The presence and amount of asparagine amino acids increase the production of acrylamide. Other amino acids reduce the amount of acrylamide and this reduction is very high for lysine amino acids [11]. In recent years, various methods such as Gas Chromatography (GC) and Liquid Chromatography (LC) were two main laboratory techniques for the determination of acrylamide. In some reports, acrylamide was measured using the GC method and Flame Ionization Detector (GC- FID), and the acrylamide derivatives were determined by GC by Electron Capture Detection method (GC-ECD) and the detection range of μg/L in liquid matrices [12]. Also, acrylamide was determined in LC method by the detection of oscillatory amperometry (LC-PAD and LOD) and it is

about μg/L in food. Recently, chromatographic methods have been used as a sensitive method to determine acrylamide. For example, for LC methods, MS-MS is used to measure acrylamide with LOD in the range of μg/kg of food [13]. The GC method with mass spectroscopy by a high-resolution GC (/HRMS) has been accompanied by an ion trap detector. Also, methods based on high-resolution mass spectrometry and flight time detector (GC-TOF-MS) or Electron Trap Detector (ECD) has been developed [14, 15]. In some cases, the acrylamide analysis using the GC MS method involves the process of acrylamide derivation and bromination of it to 2, 3-bromopropionamide [16]. Although this method is very sensitive, the derivation process requires complex and time-consuming operations. Recently, some methods have been developed to eliminate the time-consuming step of derivation and then, the acrylamide measurement is performed directly after extraction and filtered by one of the methods of GC-MS or HPLC-MS-MS [17, 18]. Despite the fact that direct acrylamide analysis using GC-MS method without dilution has an easier process, but it is rarely used. It is because of that direct acrylamide analysis using the GC-MS device are hard due to some reason such as matrix interference, lack of a low detection limit [19], and high solubility of acrylamide in water compared to the sample preparation complexity for GC organic solvents [20]. So, the growing demand for accurate determination of acrylamide has led to the development of alternative methods to measure this carcinogenic potential. Several other techniques have been developed for the detection of this compound. The electrochemical biosensors provide acceptable performance for the detection of biological molecules due to their simplicity, speed, and high sensitivity. These include amperometry [21] and voltammetry methods [22] to measure acrylamide based on the hemoglobin reactions with acrylamide. One of the used methods in food analysis is a fluorescence method used to analyze structural changes in proteins. The high selectivity of this spectroscopic method is due to the use of two types of absorption and diffusion spectra. As a result, fluorescence methods have 100 to 1000 times more sensitivity than absorption methods. However, double-spectrum spectroscopy is more expensive than absorption spectrophotometry with a single spectrum [23]. In research, acrylamide polymerization and the increased gap between quantum dots nanoparticles were used to detect acrylamide by fluorescence method in potato chips. The present acrylamide in the sample is involved in polymerization and so the fluorescence intensity increases. This method has an acceptable linear domain. Although its sensitivity is less than that of MS coupled chromatography, but it requires much less time and cost to detect fast and online acrylamide in food processing [24]. As is apparent from the acrylamide structure ($CH_2CH\text{-}CO\text{-}NH_2$), limited continuity of π electrons means that acrylamide does not have a strong chromophore for ultraviolet detection and fluorescence, and requires the use of fluorescent compounds to measure it with this method. In this study, two compounds were used as fluorescence reagents to detect the concentration of acrylamide in potato chips in real samples of 4 companies. P503 and P540 are novel amino-reactive fluorescent reagents such as Py-Dyes. The names given to them reflect that they consist of a pyrylium group attached to small aromatic moieties. Upon reaction with an amine, there is a large spectral shift in the reagent, rendering them effectively fluorogenic. The shift in absorption and the induction of fluorescence effectively eliminates background effects from any unbound dye. The labeled materials are ready to use immediately, and the labeling method is fast and easy [25]. The unreacted reagents are weakly fluorescent but present at a much higher concentration than the labeled crylamide. The unreacted reagent undergoes photobleaching at photo radiation more than an order of magnitude higher than the labeled acrylamide [26, 27]. For determination acrylamide by fluorescence based on the works done by researchers, a step of derivation and conversion to the primary amine is required, while using P503 and P540, the derivation step is not required and due to the π-π* interactions between p503 and p540 with acrylamide, the reaction takes place. Also, these reagents do not need to modify the surface, unlike quantum dots nanoparticles. They can be connected by the interaction of amino acids on the chain of acrylamide molecules containing electronegative atoms such as nitrogen and form hydrogen bonding to acrylamide. Fluorescence emission spectroscopy was also used to measure the acrylamide concentration. The obtained results were compared for acrylamide measurement using HPLC-MS method. The results showed that there is an acceptable overlap between fluorescence spectroscopy

and HPLC-MS and also the amount of acrylamide in the potato chips exceeds the allowable limited amount for the body.

EXPERIMENTAL SECTION

Chemicals and reagents

The acrylamide material with purification of 99% was purchased from Merck (Germany). Fluorescent reagents P503 and P540 were prepared from Sigma-Aldrich. Acetonitrile, methanol, N-hexane and acetate, sodium hydroxide, di-potassium hydrogen phosphate, sodium bicarbonate, potassium hexa ferrocyanide (carrez I), and zinc acetate (carrez II) were purchased from Merck (Germany). We dissolved 10.6 g of potassium hexa ferrocyanide in 100 ml of distilled water, to prepare the carrez I solution, and to prepare the carrez II solution, we mixed 21.9 g of zinc acetate solution with 3mL acetic acid and increase the volume to 100 ml. A standard solution of acrylamide was prepared with a concentration of 10 mg/L in n-hexane solvent and to prepare the desired concentrations it was diluted with n-hexane solvent. In many studies, extraction solvents such as water, n-hexane, methanol, and acetic acid have been used to extract acrylamide from food samples [28]. Depending on the food matrix, a degreasing step with an organic solvent or a disrupting step is necessary to eliminate the interference components. For protein-rich foods, carrez I and carrez II, Acetone, Ethanol, or methanol for deposition and protein removal [29]. Due to the polarization of acrylamide and the time of the extraction process with water, Carrez I, Carrez II, and acetic acid solutions were used.

Instrumental conditions

Acrylamide was measured by HPLC method by using a LC-MS-MS device series LCMS-8060 Shimadzu (triple quadrupole LCMS-8060). The Shimadzu UV-1900 absorption spectroscopy and RF-6000 fluorescence emission spectroscopy were used for measurement. The conditions of the used parameters in the HPLC method are given in Table 1. HANNAHI98131 device was used to measure pH and temperature of the samples.

Sample preparation

In brief, solid samples were first homogeneous using a conventional crushing process. To 65 g homogenized chips, 3 mL of n-hexane was added to remove the remaining chain fatty acid compounds, which could cause chromatographic analysis problems by overlapping with target analytes or blocks the chromatography column. One solution of 0.1mL of Careez I solution, 0.1mL Careez II solution, and 9.8 mL of acetic acid, (0.2 mM), were added to 1 g of homogenized sample. The mixture was mixed for 2 minutes using a mixer. The suspension was centrifuged at 5000 rpm for 10 minutes at -5°C, because at this temperature the dissolved fatty acids in n-hexane were solidified and remained as sediment after this stage, 0.5 mL of NaOH (2 mol/L) and 1 mL of NaHCO3 (2 mol/L) were added to the sample solution. In this step, the pH of the sample solution was 8. The supernatant, which is the main solution containing acrylamide, was filtered through a 0.45 μm syringe filter. In this research, at first, 3 flavors of chips including salt chips, tomatoes, and vinegar were selected from four different manufacturers. In order to obtain a precise average concentration of acrylamide, these samples were prepared over the period of two months, and each measurement was repeated 7 times, then an average value was recorded. After dilution, each sample was prepared directly for measurement by mass spectrometry, fluorescence emission, and HPLC in one day. This method was done to minimize the human error and variation type of samples.

Statistical analysis

In this study, the effective parameters such as temperature, pH, absorption, and emission wavelength were firstly studied according to the standard One Factor at a Time (OFAT) method with a standard concentration. All the parameters are considered constant in this method and only one parameter has been experimentally changed to obtain the optimal value. Then the optimized parameter is considered constant, in order to optimize other parameters. By looking at one parameter and changing one variable at a time, the results can be directly attributed to the independent variable. Design of Experiment (DOE) is a valuable experimental strategy for designing and conducting experimentation and offers a number of advantages over the OFAT. One of the important advantages of DOE is that it has the ability to discover the presence of interaction between the factors of the process [30-32]. Initially, the P540 and P503 reagent emission spectra were obtained in optimum conditions. Measurements were done at constant concentrations of 500 ng/mL and ambient temperature at different pH values.

Table 1: Optimal Conditions for HPLC Parameters.

Mobile phase	0.01 mmol/L acetic acid in 0.2% formic acid and 0.2% acetic acid in acetonitrile (98:2 v/v)
Mobile phase flow rate	0.3 ml/min
Column temperature	5 ˚C
Column	Zorbax SB-C 18 (4.6 mm× 250 mm, 5 µm)
Injection volume	40 µl
Nebulizer (N) pressure	40 psi
Drying gas flow	11 l/min
Drying gas temperature	300 ˚C
Vaporizer temperature	325 ˚C
Capillar voltage	3000 V

Schematic design of the interaction of P540 and P503 fluorescent reagents with acrylamide in solution

The structure of the P540 combination with $C_{25}H_{26}NO^+$ BF_4^-chemical formula is shown in fig.1a) and the Structure of the P503combination with $C_{21}H_{24}NO^+$ BF_4^-chemical formula is shown in fig. 1b) These are a combination of pyrylium dyes that have the property of color change and fluorescence as a result of interaction with compounds that have NH_2 groups. P540 and P503 have fluorescence emission peaks in the wavelength of 627 nm and 600 nm respectively. The presence of this peak in high wavelength is widely used in the quantitative analysis of the organic matter, including drugs, and even it is used to the tracking some organic toxins in the living body or in the food [30]. These reagents do not need to modify the surface, unlike quantum dots nanoparticles. They can be connected by the interaction of amino acids on the chain of acrylamide molecules containing electronegative atoms such as nitrogen and form hydrogen bonding to acrylamide. This causes wide variations in absorbent optical spectra and particularly the fluorescence emission spectrum. These optical changes are closely related to the concentration of acrylamide present in the environment, which will be further discussed in more detail.

Investigation of the pH effect on the P540and P503 absorption spectra

As shown in Fig. 2a and Fig. 2b, by increasing pH, not only absorption wavelength is shifted to the larger domain (Red Shift), but also the absorption rate increases [33]. According to the purpose of detecting and determining the concentration of acrylamide in the next steps, the optimal pH 8 was chosen, because it is less intense at lower pHs. At pH higher than 8, the absorption intensity of P540 and P503 reagents is higher, but the probability of acrylamide decomposition is high, so the optimum temperature parameter is only obtained at this pH, as shown below. The P540 and P503 reagents at pH= 8 have an absorption peak of 538 nm and 505 nm respectively.

Investigation of the effect of different temperatures on P540and P503 absorption spectra in pH = 8

The absorption intensity of P540 and P503 reagents also increases by increasing temperature, with regard to the absorption spectra shown in Fig. 3a) and Fig. 3b). It should be noted that the best optimum temperature could be chosen at 160 °C, but the environment (between 20-30 °C) temperature consider optimum, due to the probable constraints for introducing an industrial method for measuring acrylamide, and also the limitation of absorption and emission devices. In the absence of these restrictions, a higher temperature can be selected.

Investigation of the effect of pH on the fluorescence spectra of P540 and P503 reagents

Fluorescence spectroscopy measurements were made at different pH, as in the UV-Vis absorption spectroscopy, which is according to the OFAT method and at a standard concentration of 500 ng/mL. As shown in Fig. 4a and Fig. 4b, the optimum pH for determination is 12, but not only the acrylamide is decomposed at this pH, but also shows a large blue shift than pH= 8 [34]. According to the above, pH = 8 is the best choice. The P540 and P503 reagents show emission peaks at 630 nm and 550 nm respectively.

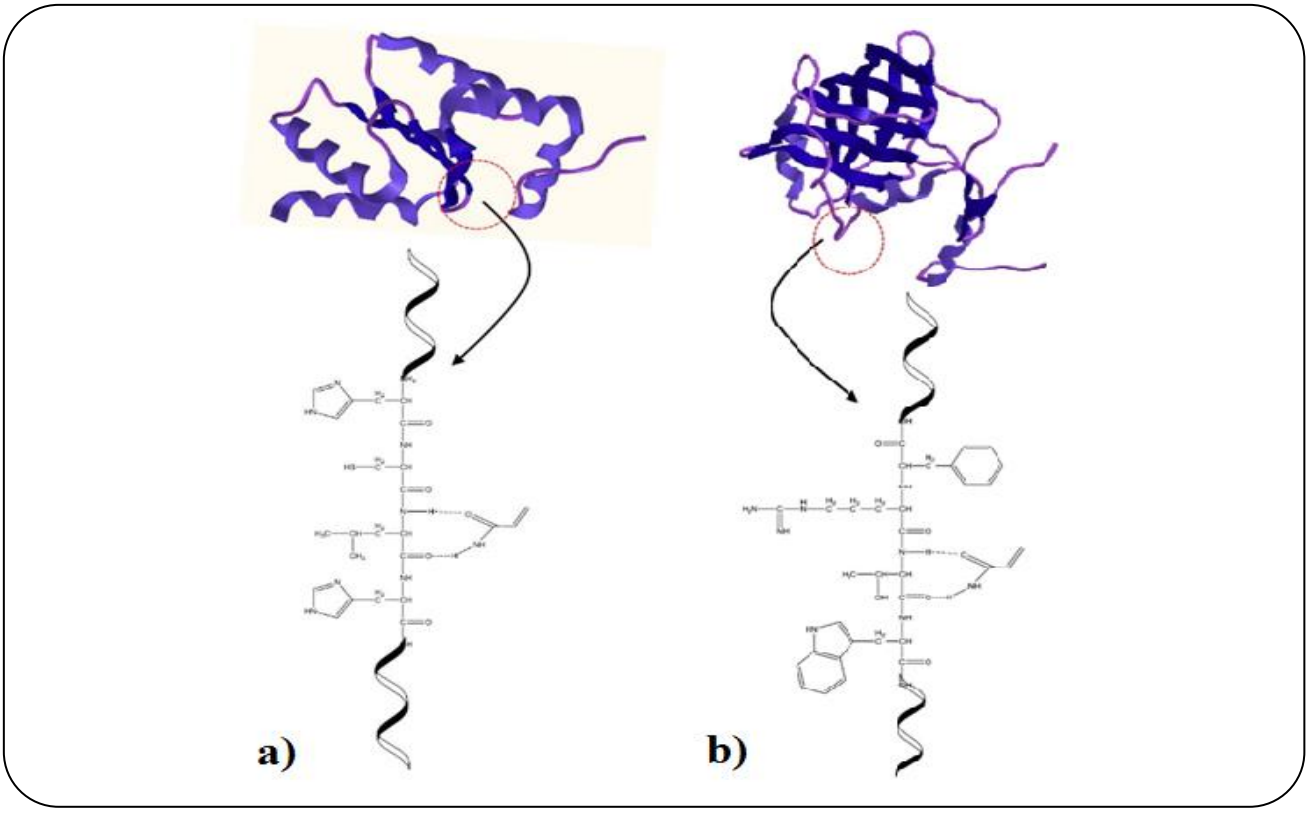

Fig. 1: Acrylamide interconnection with a) P540 reagent, b) P503 reagent.

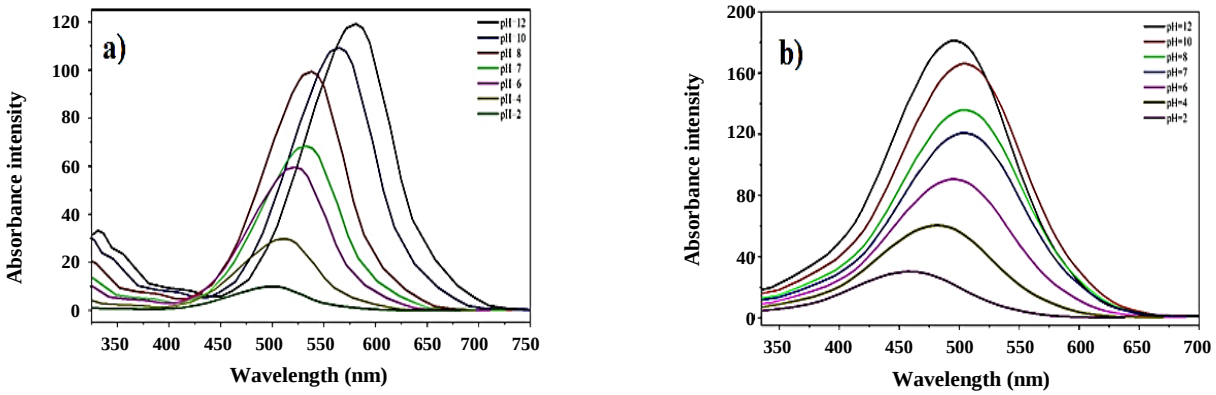

Fig. 2: UV-Vis absorption spectrum in different pH, a) P540 and b) P503.

Investigation of the effect of temperature on fluorescence emission spectra of P540 and P503 reagents at pH = 8

According to the spectra shown in Fig. 5a) and Fig. 5b), the intensity of emission increases by increasing temperature. It should be noted that the best optimum temperature could be chosen at 160 °C, but the environment (between 20-30°C) temperature is considered optimum, due to the probable constraints of introducing an industrial method for measuring acrylamide and also the limitation of absorption and emission devices. In the absence of these restrictions, a higher temperature can be selected.

Investigating the effect of pH on P540 and P503 fluorescence spectra in the presence of acrylamide

So far, to measure the acrylamide concentration, parameters optimization was initially carried out using

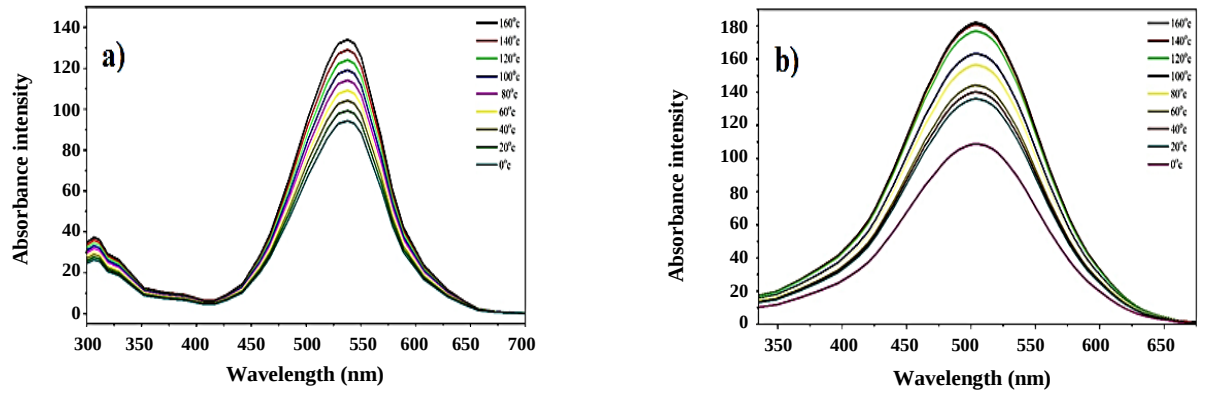

Fig. 3: UV-Vis absorption spectrum of reagents at pH = 8 and different temperatures a) P540 and b) P503.

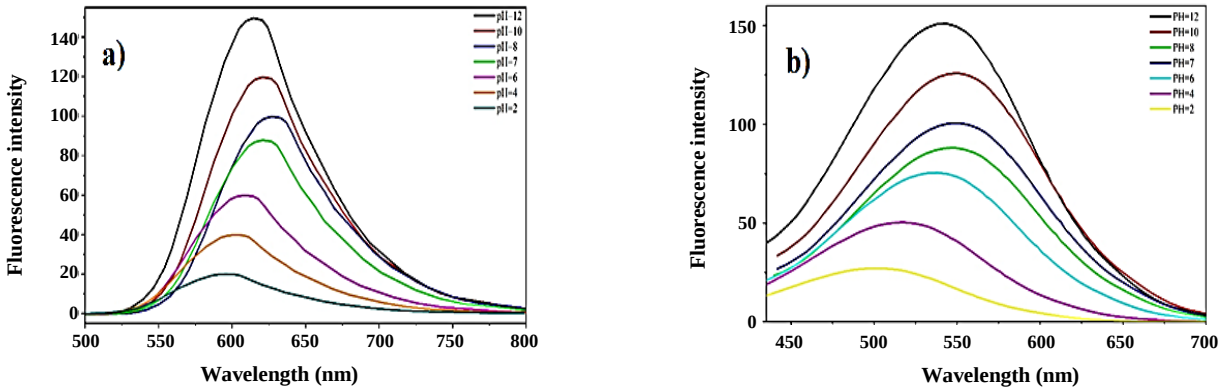

Fig. 4: Fluorescence spectra of reagents in different pH, a) P540 and b) P503.

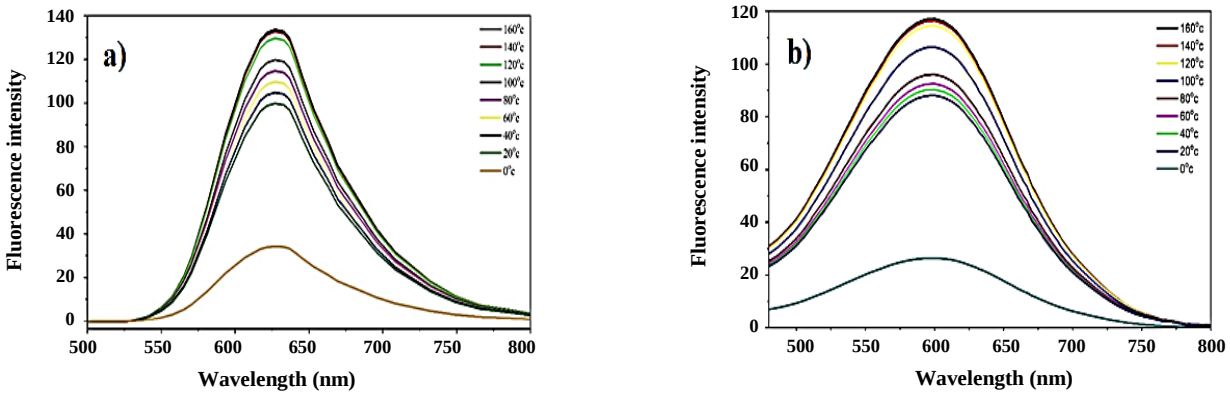

Fig. 5: Fluorescence spectra at pH= 8 and different temperatures, a) P540 and b) P503.

standard concentration and based on the OFAT method. As for the P540 and P503 emission spectra, the best pH is pH= 8 to measure acrylamide, which is shown in Fig. 6a) and Fig. 6b). But, significant changes such as the peak broadening are clearly visible when acrylamide bonding to the P540 and P503 surface, and also a curvature of the emission spectrum close to the ultraviolet region, and also a bluish shift occurred in the fluorescence emission spectrum. The reason is the Photo-induced Electron Transfer (PET) phenomenon. This phenomenon has occurred due to

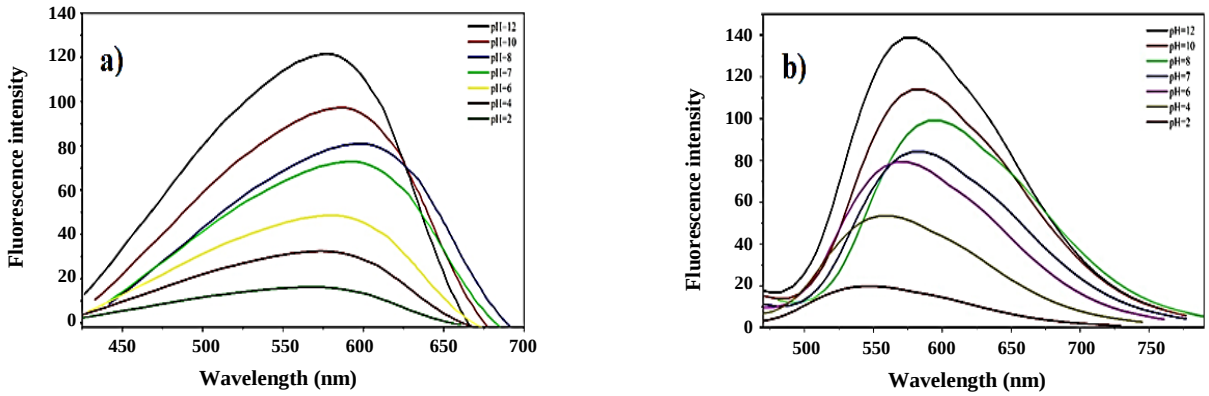

Fig. 6: Fluorescence emission spectroscopy of reagents in the presence of acrylamide at different pH and at a standard concentration of 500 ng/ml, a) P540 and b) P503.

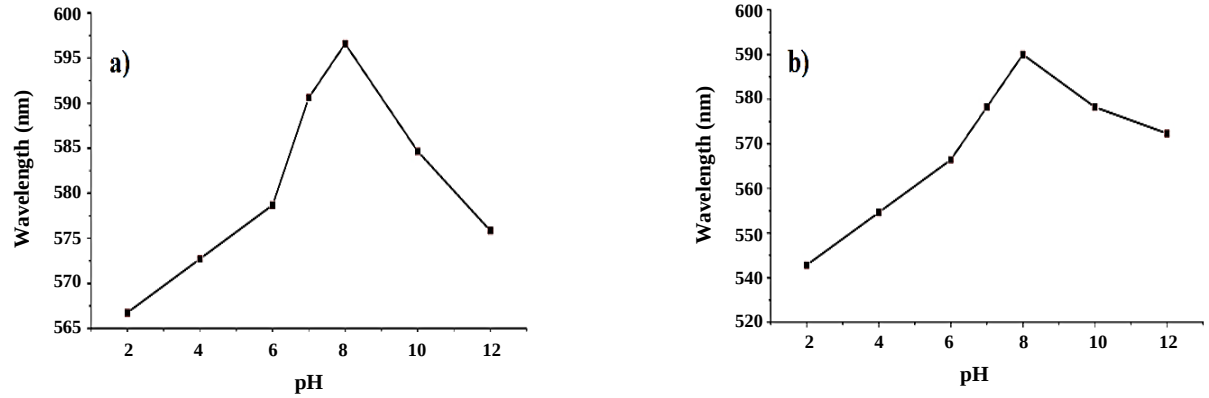

Fig. 7: Maximum wavelength changes of the fluorescence emission spectrum at different pH in the presence of acrylamide at a concentration of 500 ng/ml, a) P540 and b) P503.

the transfer of the nonbonding electron pair of the amide group after receiving the wavelength. The fluorescence emission spectrum of the P540 in the presence of acrylamide and at pH= 8 shows a peak emission at 605 nm and the emission spectrum of the P503 in the presence of acrylamide and at pH= 8 shows a peak emission at 595 nm.

Also, as seen in Fig. 7a) and Fig. 7b), the trend of these changes can be seen in the graph in terms of emission wavelengths, which the highest emission wavelength is at pH= 8. The highest emission wavelength is very important in quantity identification. The emission spectrum with the lowest absorption range has an acceptable sensitivity to its lower wavelengths, which is because of two reasons; firstly, due to the absence of the emission spectrum of conventional impurities at high wavelengths and, secondly, the higher emission wavelength spectrum of matter.

Investigation of the effect of temperature on the fluorescence emission spectrum of P540 and P503 in the presence of acrylamide

The temperature parameter in the fluorescence spectroscopy of P540 and P503 in the presence of a standard concentration of acrylamide has exactly the same effect as the alone fluorescence emission spectrum of the P540 and P503, and their intensity has increased by increasing temperature, as shown by the spectra related to these effects in Fig. 8a and Fig. 8b. It should be noted that the temperature has been evaluated at optimal pH= 8. It is also possible to observe these changes in Figs. 9a and 9b, in terms of variation intensity versus temperature.

Measurement of different concentrations of acrylamide and calibration curve

In Fig. 10a and Fig. 10b, the standardized curve

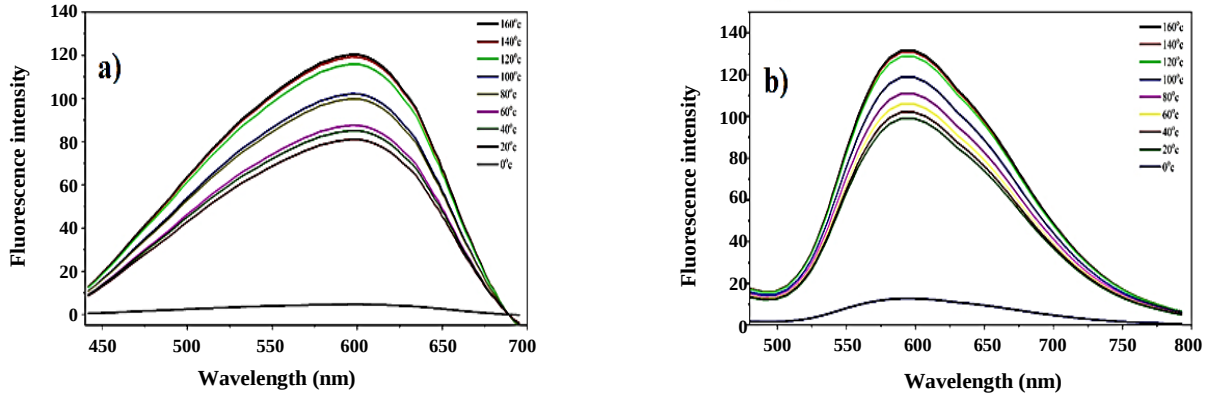

Fig. 8: Fluorescence emission spectra at different temperatures in the presence of acrylamide at a concentration of 500 ng/ml and pH= 8, a) P540 and b) P503.

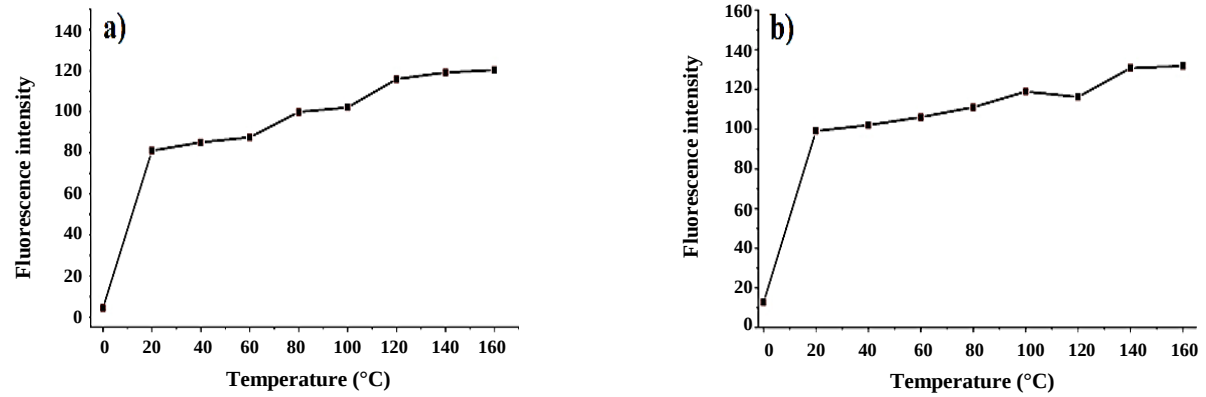

Fig. 9: Trend of intensity change of the fluorescence emission spectrum at different temperatures versus to the emission intensity in the presence of acrylamide at a standard concentration of 500 ng/ml and pH= 8, a) P540 and b) P503.

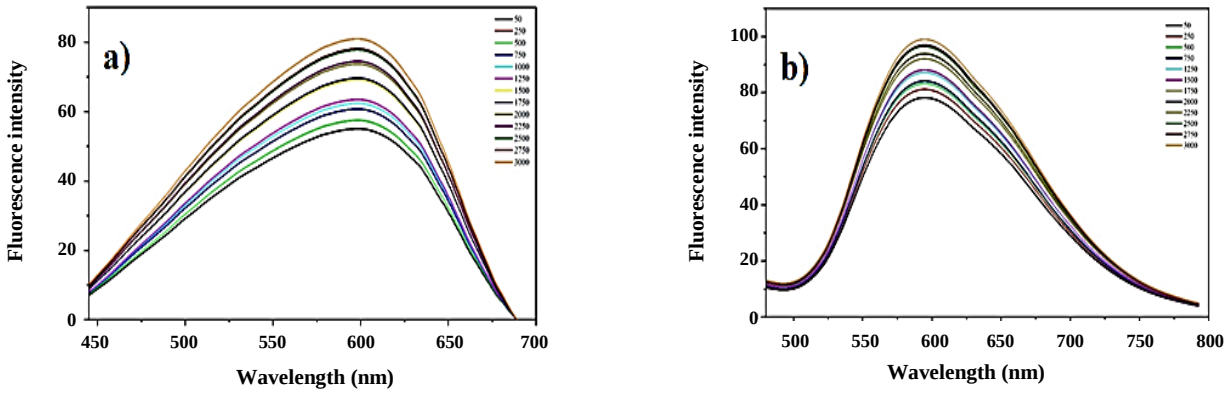

Fig. 10: Trend of intensity change of the fluorescence emission spectrum at pH= 8 and at standard concentrations of acrylamide 500-3000 ng/ml, a) P540 and b) P503.

fluorescence emission spectra of the standard concentrations of acrylamide with a standard concentration of 3000-500 ng/mL were presented using fluorescence spectroscopy and in the presence of P540 and P503 reagents in optimum pH and temperature. As shown in the graph, the emission intensity increased by increasing acrylamide concentration, which

Table 2: Measured values of acrylamide concentrations in potato chips using fluorescence spectroscopy and HPLC/MS methods.

Acrylamide concentrations (ppb)										
Company	Type of chips	Average of the first month			Average of the second month			Total average		
		HPLC	P540	P503	HPLC	P540	P503	HPLC	P540	P503
C_1	Salty	1567	1575	1575	1556	1563	1576			
	Tomatoes	1712	1718	1719	1732	1742	1743	1705.5	1714.1	1717.5
	Vinegar	1845	1857	1860	1821	1830	1832			
C_2	Salty	1784	1791	1789	1743	1749	1740			
	Tomatoes	1932	1940	1944	1965	1975	1979	1924.8	1934.3	1933.8
	Vinegar	2051	2065	2061	2074	2086	2085			
C_3	Salty	1613	1621	1617	1676	1680	1683			
	Tomatoes	1847	1859	1859	1821	1832	1834	1812.7	1822.6	1822.8
	Vinegar	1939	1955	1953	1981	1989	1991			
C_4	Salty	1933	1941	1940	1919	1937	1928			
	Tomatoes	2103	2109	2117	2081	2094	2094	2217.9	2214.7	2214
	Vinegar	2576	2590	2587	2603	2617	2816			

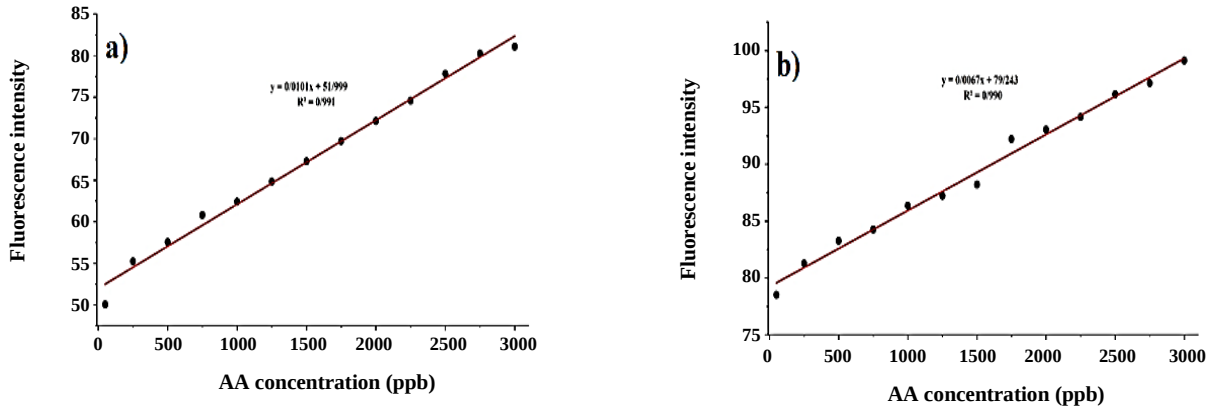

Fig. 11: Standard calibration curve of the fluorescence emission spectrum at pH = 8 and at standard concentrations of acrylamide 500-3000 ng/ml, a) P540 and b) P503.

is a result of the interaction between acrylamide and fluorescent reagents P540 and P503. These spectra are also presented in Fig. 11a) and Fig. 11b) as standard calibration curves. The results show that the measurement of acrylamide concentration is linear in mentioned concentration range by using this method and the method has acceptable sensitivity. In order to measure the figure of merit of the method, parameters such as Dynamic Linear Range (DLR), repeatability of the method, Limit of Detection (LOD), and Limit of Quantification (LOQ) were obtained. The calibration curve is linear in the range of 500-3000 ng/mL with a correlation coefficient (R^2) of 0.991 and 0.0990 for P540 and P503 respectively. The Limit of Detection (LOD) and the Limit of Quantification (LOQ) of the method are 50 and 150 ng/mL, respectively.

Determination of acrylamide in the real sample of potato chips

In Table 2, the measured concentrations of acrylamide concentrations from the real sample in 4 commercial brands of chips in the presence of P540 and P503 reagents

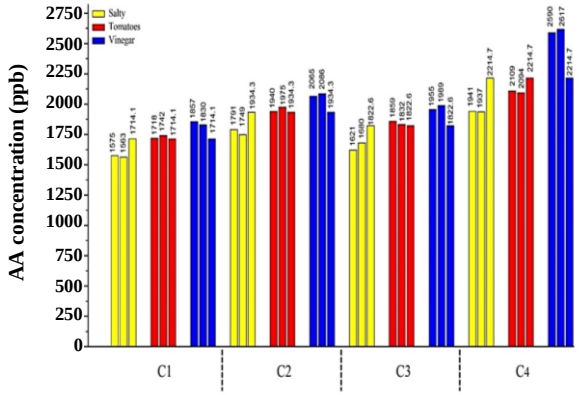

Fig. 12: Comparison of acrylamide content in potato chips samples from 4 different companies in the first, second, and average of two months using fluorescence spectroscopy and in the presence of P540.

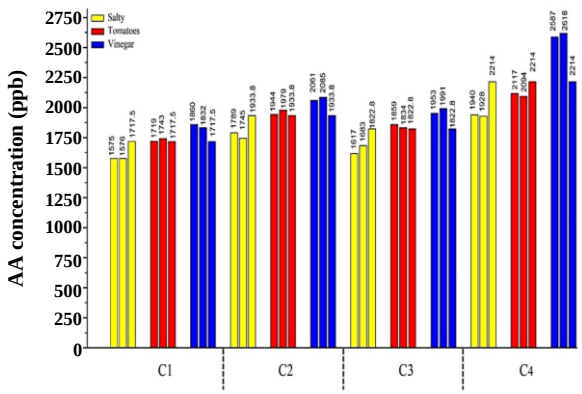

Fig. 13: Comparison of acrylamide content in potato chips samples from 4 different companies in the first, second and average of two months using fluorescence spectroscopy and in the presence of P503.

after preparation, the sample is presented by fluorescence emission spectroscopy. In addition to the spectrophotometric emission method, the concentrations were compared by HPLC-MS method. In all measurements, the spectroscopy has been repeated an average of 7 times, and the obtained average is presented in Table 2. Fig. 12 and Fig. 13, show the comparison between acrylamide values in chips of the manufacturing companies in different months. In these graphs, each column with the same color from right to left are corresponding to the first month, the second month, and the average of two months, and C1, C2, C3, and C4 are samples from 1 to 4 companies.

Considering the values obtained from all the measurements, and considering that the World Health Organization (WHO) agreed that the average intake of acrylamide by food at the national level ranged from 0.3 to 2 μg/kg of body weight per day in the general population, all measured samples are higher than the limit. Table 3 shows the amount of acrylamide for a 70 kg person with the present methods in this study for a 65 grams chips package.

CONCLUSIONS

In this work, acrylamide was identified in real samples using two fluorescent reagents P503 and P504 separately. For this purpose, true samples of chips and potatoes were first prepared according to the reference method, and this true sample was used to identify acrylamide in four chips. In order to exact comparison of all the used methods, a true sample was used. The HPLC method was selected reference method for comparison and the fluorescence method was compared with the P503 and P540 detectors and then accurately measured. The P540 and P503 proteins have a fluorescence emission peak at 627 and 600 nm, respectively. There is a large application in the quantitative analysis of organic matter, including drugs, and even the interception of some organic toxins in the body of living organisms or food, which is because of the presence of the high wavelength peak. The optics variations are in agreement with the acrylic amide concentration in the environment. The parameters optimization was done using the OFAT method. pH= 8 and the ambient temperature was chosen as the optimum temperature. The fluorescence emission spectrum for P540 and P503 was observed at 597 nm and 593 nm, respectively in the presence of acrylamide. Totally from the fluorescence emission spectroscopy, the observed peak wavelengths of three detectors show a slight blue shift in the presence of acrylamide, which is due to the occurrence of PET phenomena from acrylamide to the detectors. The measured errors in the used method for P540 and P503 were 0.99% of the HPLC method. This method can be a good alternative to the HPLC method, due to the poor error, cost-effectiveness, reduced the measuring time, as well as suitability parameters such as linear range (500-3000 ng/mL) (DLR), repeatability (RSD), detection limit (LOD) and measurement limit (LOQ). The results show that all samples had a higher amount of acrylamide than the limit, which company 1 having the lowest interval with the limit (about1.59 times of limit) and company 4 having the highest interval with the limit (about2.06 times of limit).

Table 3: Comparison of Acrylamide Concentration to the allowable Limit for a 70 Kg person during 1 Day for Each company.

Method	Company	Obtained concentration(µg)	Allowable concentration(µg)	Uper than allowable limit(%)
HPLC-MS	C1	110.86	70	1.58
	C2	125.12	70	1.79
	C3	117.83	70	1.68
	C4	144.16	70	2.06
Fluorescence by using P540 reagent	C1	111.42	70	1.59
	C2	125.73	70	1.80
	C3	118.47	70	1.69
	C4	143.96	70	2.06
Fluorescence by using P503 reagent	C1	111.64	70	1.59
	C2	125.70	70	1.80
	C3	118.48	70	1.69
	C4	143.91	70	2.06

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REFERENCES

[1] Arvanitoyannis I.S., Dionisopoulou N., Acrylamide: Formation, Occurrence in Food Products, Detection Methods, and Legislation, *Critical Reviews in Food Science and Nutrition*, **54(6)**: 708-733 (2014).

[2] Stadler R.H., Acrylamide Formation in Different Foods and Potential Strategies for Reduction, In: "Chemistry and Safety of Acrylamide in Food", Springer, Boston MA., (pp. 157-169), (2005).

[3] Gökmen V., "Acrylamide Formation Mechanisms, Acrylamide in Food: Analysis, Content and Potential Health Effects", Elsevier, London, 1-17, (2016).

[4] Hu Q., Xu X., Li Z., Zhang Y., Wang J., Fu Y., Li Y. Detection of Acrylamide in Potato Chips Using a Fluorescent Sensing Method Based on Acrylamide Polymerization-Induced Distance Increase Between Quantum Dots, *Biosensors and Bioelectronics*, **54**: 64-71 (2014).

[5] Friedman M., Chemistry, Biochemistry, and Safety of Acrylamide. A Review, *Journal of Agricultural and Food Chemistry*, **51(16)**: 4504-4526 (2003).

[6] Dybing E., Farmer P.B., Andersen M., Fennell T.R., Lalljie S.P.D., Müller D.J.G., Scimeca J.A., Human Exposure and Internal Dose Assessments of Acrylamide in Food, *Food and Chemical Toxicology*, **43(3)**: 365-410, (2005).

[7] Cagliero C., Ho T.D., Zhang C., Bicchi C., Anderson J.L., Determination of Acrylamide in Brewed Coffee and Coffee Powder Using Polymeric Ionic Liquid-Based Sorbent Coatings in Solid-Phase Microextraction Coupled to Gas Chromatography-Mass Spectrometry, *Journal of Chromatography A*, **1449**: 2-7 (2016).

[8] Claeys W., Baert K., Mestdagh F., Vercammen J., Daenens P., De Meulenaer B., Huyghebaert A., Assessment of the Acrylamide Intake of the Belgian Population and the Effect of Mitigation Strategies, *Food Additives and Contaminants*, **27(9)**: 1199-1207 (2010).

[9] Claeys W.L., De Vleeschouwer K., Hendrickx M.E., Kinetics of Acrylamide Formation and Elimination During Heating of an Asparagine− Sugar Model System, *Journal of Agricultural and Food Chemistry*, **53(26)**: 9999-10005, (2005).

[10] Pedreschi, F., Kaack, K., Granby, K. Reduction of Acrylamide Formation in Potato Slices During Frying, *LWT-Food Science and Technology*, **37(6)**: 679-685 (2004).

[11] Arvanitoyannis I. S., Dionisopoulou N., Acrylamide: Formation, Occurrence in Food Products, Detection Methods, and Legislation, *Critical Reviews in Food Science and Nutrition*, **54(6)**: 708-733 (2014).

[12] Elbashir A.A., Omar M.M.A., Ibrahim W.A.W., Schmitz O.J., Aboul-Enein H.Y., Acrylamide Analysis in Food by Liquid Chromatographic and Gas Chromatographic Methods, *Critical Reviews in Analytical Chemistry*, **44(2)**: 107-141 (2014).

[13] Hoenicke K., Gatermann R., Harder W., Hartig L., Analysis of Acrylamide in Different Foodstuffs Using Liquid Chromatography–Tandem Mass Spectrometry and Gas Chromatography–Tandem Mass Spectrometry, *Analytica Chimica Acta*, **520(1-2)**: 207-215 (2004).

[14] Sun S.Y., Fang Y., Xia Y.M., A Facile Detection of Acrylamide in Starchy Food by Using a Solid Extraction-GC Strategy, *Food Control*, **26(2)**: 220-222 (2012).

[15] Kim S.H., Hwang J.H., Lee K.G., Analysis of Acrylamide Using Gas Chromatography-Nitrogen Phosphorus Detector (GC-NPD), *Food Science and Biotechnology*, **20(3)**: 835-839 (2011).

[16] Dunovská L., Čajka T., Hajšlová J., Holadová K., Direct Determination of Acrylamide in Food by Gas Chromatography–High-Resolution Time-of-Flight Mass Spectrometry, *Analytica Chimica Acta*, **578(2)**: 234-240 (2006).

[17] Soares C.M.D., Alves R.C., Casal S., Oliveira M.B.P., Fernandes J.O., Development and Validation of a Matrix Solid- Phase Dispersion Method to Determine Acrylamide in Coffee and Coffee Substitutes, *Journal of Food Science*, **75(3)**: 57-63 (2010).

[18] Lineback D.R., Coughlin J.R., Stadler R.H., Acrylamide in Foods: A Review of The Science and Future Considerations, *Annual Review of Food Science and Technology*, **3**: 15-35 (2012).

[19] Mastovska K., Lehotay S.J., Rapid Sample Preparation Method for LC-MS/MS or GC− MS Analysis of Acrylamide in Various Food Matrices, *Journal of Agricultural and Food Chemistry*, **54(19)**: 7001-7008 (2006).

[20] Castle L., Eriksson S., Analytical Methods Used to Measure Acrylamide Concentrations in Foods, *Journal of AOAC International*, **88(1)**: 274-284 (2005).

[21] Zhang S., Wright G., Yang Y., Materials and Techniques for Electrochemical Biosensor Design and Construction, *Biosensors and Bioelectronics*, **15(5-6)**: 273-282 (2000).

[22] Batra B., Lata S., Sharma M., Pundir C.S., An Acrylamide Biosensor Based on Immobilization of Hemoglobin onto Multiwalled Carbon Nanotube/Copper Nanoparticles/Polyaniline Hybrid Film, *Analytical Biochemistry*, **433(2)**: 210-217 (2013).

[23] Murkovic M., Acrylamide in Austrian Foods, *Journal of Biochemical and Biophysical Methods*, **61(1-2)**: 161-167 (2004).

[24] Hu Q., Xu X., Li Z., Zhang Y., Wang J., Fu Y., Li Y., Detection of Acrylamide in Potato Chips Using a Fluorescent Sensing Method Based on Acrylamide Polymerization-Induced Distance Increase Between Quantum Dots, *Biosensors and Bioelectronics*, **54**: 64-71 (2014).

[25] Douglas B. Craig, Bianca K. Wetzl, Axel Duerkop, Otto S. Wolfbeis, Determination of Picomolar Concentrations of Proteins Using Novel Amino Reactive Chameleon Labels and Capillary Electrophoresis Laser-Induced Fluorescence Detection, Electrophoresis, (2005).

[26] Emily H. Turner, Jane A. Dickerson, Lauren M. Ramsay, Kristian E. Swearingen, Roza Wojcik, Norman J. Dovichi, Reaction of Fluorogenic Reagents with Proteins III. Spectroscopic and Electrophoretic Behavior of Proteins Labeled with Chromeo P503, *J. Chromatogr. A.*, **1194(2)**: 253–256 (2008).

[27] Shahraki S., Shojaei S., Shojaei S., Inhibitory Role of β-Casein on the α-Synuclein Aggregation Associated with Parkinson's Disease *in vitro*, *Int. J. Pept. Res. Ther.*, **24**: 179 (2018).

[28] Bermudo E., Moyano E., Puignou L., Galceran M.T., Liquid Chromatography Coupled to Tandem Mass Spectrometry for the Analysis of Acrylamide in Typical Spanish Products, *Talanta*, **76(2)**: 389-394 (2008).

[29] Bagdonaite K., Derler K., Murkovic M., Determination of Acrylamide During Roasting of Coffee, *Journal of Agricultural and Food Chemistry*, **56(15)**: 6081-6086 (2008).

[30] Shokri A., Rabiee F., Mahanpoor K., Employing a Novel Nanocatalyst (Mn/Iranian hematite) for Oxidation of SO_2 Pollutant in Aqueous Environment, *Int. J. Environ. Sci. Technol*, **14 (11)**: 2485–2494 (2017).

[31] Mohadesi M., Shokri A., Evaluation of Fenton and Photo-Fenton Processes for the Removal of p-Chloronitrobenzene in Aqueous Environment Using Box-Behnken Design Method, *Desalination and Water Treatment Science and Engineering*, **81**: 199-208 (2017).

[32] Shokri A., Employing Electrocoagulation for the Removal of Acid Red 182 in Aqueous Environment by Using Box-Behenken Design Method, *Desalination and Water Treatment Science and Engineering*, **115**: 281-287 (2018).

[33] Shokri A., Mahanpoor K., Soodbar D., Degradation of 2-Nitrophenol from Petrochemical Wastewater by UV/$NiFe_2O_4$/clinoptilolite Process, *Fresenius Environmental Bulletin*, **25(2)**: 500-508 (2016).

[34] Yarmoluk S.M., Kostenko A.M., Dubey I.Y., Interaction of Cyanine Dyes with Nucleic Acids. Part 19: New Method for the Covalent Labeling of Oligonucleotides with Pyrylium Cyanine Dyes, *Bioorganic & Medicinal chemistry Letters*, **10(19)**: 2201-2204 (2000).