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RESEARCH ARTICLE

Quantitative Analyses of Glyoxal and Methylglyoxal Compounds in French Fry Samples by HPLC Using 4-Nitro-1,2-Phenlenediamine as A Derivatizing Reagent

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HIGHLIGHTS

> The study presents an accurate HPLC method to determine AGE concentration in foods.
> Two major AGE precursors were determined; glyoxal and methylglyoxal.
> High-heat processed French fries were high in AGE content.
> High-fat foods such as French fries were high in AGE content.
> The study presents data that can be used to quantify dietary AGE intake.

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ABSTRACT

Nowadays, there is a rising trend in processed food consumption. 1,2-Dicarbonyl compounds, glyoxal (GO) and methylglyoxal (MGO), can be formed during thermal processing by Maillard reaction and oxidation of proteins and fats as precursors of advanced glycation end products (AGEs) in foods. The purpose of the present study was to determine the precursors of advanced glycation end products, GO and MGO, in French fries, which have a large place in today's modern diet and to evaluate their effects on health. The samples were purchased from different fast-food restaurants in Istanbul, Turkey. The amounts of glyoxal and methylglyoxal in these foods were determined by HPLC using 4-nitro-1,2-phenlenediamine as a pre-column derivatizing reagent. The amounts of GO and MGO were determined in French fry samples. The measured amounts of GO ranged between 2 and 428 μg/100 g in samples. However, the measured amounts of MGO ranged between 122 and 340 μg/100 g. French fries contained higher levels of MGO than GO. The health problems related to AGEs can be reduced with an AGE-restricted diet. Therefore, it is suggested to reduce the consumption of foods rich in AGEs to reduce high AGE intake in the diet.

Contents

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1. Introduction

Today, most of the foodstuffs consumed by industrialized societies are processed. Modern diets are generally thermal-processed and consequently include high amounts of advanced glycation end products (AGEs). AGEs are compounds that occur endogenously in the body and exogenously in foodstuffs, particularly during heat processing. Unlike endogenous AGEs, exogenous (dietary) AGEs are formed at a much higher rate [1]. AGEs can be formed by Maillard reaction (MR), oxidation of proteins and fats in food processing. Maillard reactions are initiated by the non-enzymatic reaction between a carbonyl group of reducing sugars and an amino group of proteins. MR products are formed during food processing, and these products contribute to improving the appearance and sensory properties of foodstuffs [2].

Glyoxal (GO) and methylglyoxal (MGO), α-dicarbonyl (α-DC) compounds, are reactive intermediate products formed during the processing of foods such as frying, baking, roasting, and storage. GO and MGO are formed by lipid peroxidation, sugar autoxidation, and microbial fermentation and are precursors of AGEs [3, 4]. These α-DC compounds react by amino groups of proteins to form final AGEs. Reaction with a lysine residue of protein results in the formation of N-ε-carboxymethyllysine (CML) from GO and N-ε-carboxyethyllysine (CEL) from MGO [5]. The CML levels in foods increase with increasing amounts of fats and proteins. High-fat containing foodstuffs such as olive oil, butter, cookies, and biscuits contain high levels of CML [2].

The formation of AGEs as endogenous is part of human metabolism. This reaction occurs as the glycation is initiated by the attachment of reducing sugars to amino groups of proteins and nucleic acids to generate unstable Schiff bases. This Schiff bases undergo unstable Amadori rearrangements and convert to more stable Amadori products. Finally, these products convert to the final, non-reversible, and highly reactive carbonyl compounds such as GO, MGO, and 3-deoxyglucosone. These carbonyl compounds can occur during glucose autoxidation, lipid peroxidation, and the polyol pathway in human metabolism [6]. İncreased concentrations of GO, MGO, and 3-deoxyglucosone are found in the plasma of type 2 diabetes mellitus (T2DM) patients. MGO and GO react with insulin to produce the final AGEs. The products can cause insulin resistance and reduce cellular glucose uptake [7]. The glucose concentration is high in diabetic patients due to the low insulin secretion from the pancreas [8]. Increased glucose reacts with body proteins two or three times than those of healthy humans to produce highly reactive GO and MGO [7].

The accumulation of AGEs can cause some chronic diseases such as diabetic complications, insulin resistance, cardiovascular disease, Alzheimer's, hypertension, Parkinson's disease, kidney disease, arthritis, nephropathy, multiple sclerosis, renal failure, and aging [1, 6]. In the circulation, the excessive amount of AGEs causes inflammation and oxidative stress through binding to the cell surface and cross-linking with proteins. There is strong evidence that dietary uptake of AGEs associates with serum AGE levels [9]. Studies reported that an AGE-restricted diet decreases serum AGE levels in individuals with T2DM [10]. A daily 4000-24,000 kU/day AGE intake was reported for healthy individuals and individuals suffering from chronic disorders such as kidney disease and diabetes mellitus. The daily CML and CEL intake was predicted depend on a food frequency questionnaire for people suffering from cardiovascular disease and diabetes mellitus. The mean CML and CEL intake were 3.1 and 2.32 mg/day, respectively [1].

HPLC is the favored analytical technique to determine the α-DCs. Pre-column derivatization is required because there is no chromophoric group in α-DC structures [11]. The derivatization reagents of 4-(2,3-dimethyl-6-quinoxalinyl)-1,2-benzenediamine, o-phenlenediamine (OPD) [12], and 4 nitro-1,2-phenlenediamine [13] can produce UV sensitive quinoxaline structures with α-DCs. As can be seen, different analytical methodologies containing different extraction methods, reagents, and instrumentation are used for GO and MGO investigation.

There is evidence that French fries and other snack foods cause obesity and other chronic diseases in children and adolescents [14–16]. In the United States, consumption of snack foods contributed to 30% of the calorie intake of children between the ages of 2 and 5 in between 1977 and 1996 [17]. According to the families' declaration in a survey in Turkey, 8.7% of children aged 7-8 consumed snack foods such as chips and popcorn every day [14].

French fries examined in this study are heat-treated and contain high amounts of fat. Generally, α-DCs compounds, GO and MGO, can be formed in lipid- and sugar-rich foods. These intermediate compounds react with proteins to form final harmful AGEs, and it is essential to identify these precursors in foods to predict the final AGEs. In the literature, there is limited data on the precursors of AGEs in French fries. This investigation aimed to determine the amount of GO and MGO in French fries and to assess their effects on human health.

2. Material and Methods

The stages of the study are summarized in Figure 1.

2.1. Chemicals

Glyoxal, methylglyoxal, methanol, sodium acetate, 4-nitro-1,2-phenlenediamine, acetonitrile, fructose, glucose, and sucrose were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Sampling

In this study, seven different French fries were purchased from various fast-food restaurants in Istanbul, Turkey.

2.3. Extraction and Derivatization of GO and MGO

The extraction method for GO and MGO in foods described by Mahar et al. (2010) was performed with some modifications [13]. First, all samples were homogenized with a blender. Then, 5 g of each French fry was weighed into a 50 mL plastic falcon tube, and 20 mL methanol was added. Following, the sample was extracted with an ultra-thorax homogenizer for 2 min and centrifuged for 5 min at 8000 rpm. Next, 0.5 mL of liquid sample was taken into a 10

mL glass tube, and 1 mL sodium acetate buffer (0.1 M, pH: 3) was added. Afterwards, 0.5 mL of derivatization solution (4-nitro-1,2-phenlenediamine in 1% methanol) was added. The mixture was incubated at 70 °C for 20 min and then filtered with a cellulose acetate filter (0.45 µm), injected into the HPLC (Figure 1).

2.4. HPLC Determination of GO and MGO

The GO and MGO precursors of AGE were detected by High-performance liquid chromatography according to the analytical method described by Mahar et al. (2010) [13] with some modifications.

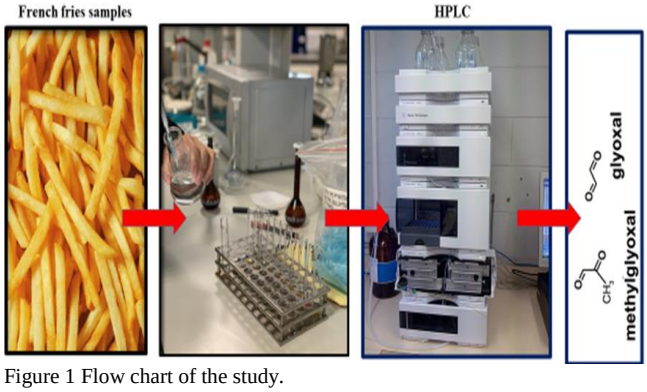

Figure 1 Flow chart of the study.

The Shimadzu LC 20AT pump with a Shimadzu SPD-20A UV/VIS detector (Shimadzu Corporation, Kyoto, Japan) was performed in the analysis. The mobile phase consists of methanol:water:acetonitrile (42:56:2, v/v/v). The wavelength was set at 255 nm. The GO and MGO were separated with an Inertsil ODS-3 column with a flow rate of 1 mL/min. The oven temperature of the column was set at 30°C.

The GO and MGO amount of each sample was based on the mean amount of at least three analyses per sample and shown as AGE µg/100 g food. The results were given as mean ± standard deviation.

3. Results and Discussion

HPLC is one of the most appropriate and accurate analytical methodologies for determining the AGE precursors in foods. The HPLC chromatogram of French fries is shown in Figure 2. As seen in the chromatogram, GO and MGO were well-separated using the HPLC method. Figure 3 shows the formation process of AGE precursors in French fries. The samples of French fries were examined to determine the amounts of GO and MGO. The measured amounts of GO and MGO in French fries are given in Table 1. The measured amounts of GO ranged between 2 and 428 µg/100 g in samples. However, the measured amounts of MGO ranged between 122 and 340 µg/100 g. In the results, French fries contained higher levels of MGO than GO.

Comparing the amounts of GO and MGO in the samples, MGO was predominant in 5 of 7 samples. The dietary AGE level was high, except for one sample with negligible GO content (2 µg/100 g, for sample 4). The difference may be due to the type of fat, cooking temperature, sample preparation, processing conditions, and other ingredients.

Table 1 GO and MGO content in French fries.

Sample No	GO (µg/100 g)	MGO (µg/100 g)
1	428±5	340±20
2	96±3	188±5
3	11±2	122±3
4	2±1	162±6
5	31±3	122±6
6	304±7	281±5
7	57±3	154±5

Values are mean±SD, n=3. GO, glyoxal; MGO, methylglyoxal.

It is now well known that the modern diet is a great source of AGE. Many foodstuffs in modern diets are cooked or heat-processed to increase the appearance, color, and flavor as well as for safety and convenience. Particularly, frying, grilling, roasting, broiling, and searing propagate and accelerate new AGE formations [2]. In previous research, Patron (1951) studied the nutritional, chemical, and safety characteristics of Maillard reactions in foods and has revealed that Maillard reactions were also responsible for the generation of aroma in fried potatoes [18].

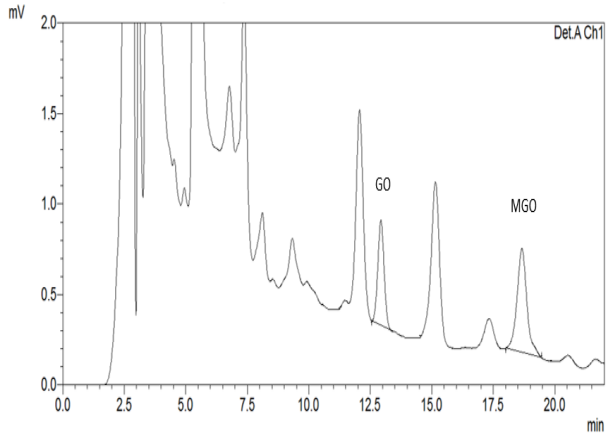

Figure 2 HPLC chromatogram of GO and MGO in French fries (for sample 6).

AGE formation during heat processing, as observed in many studies [1, 2, 19], was confirmed in this study. High AGE contents were detected in French fries, and the findings were in good accordance by Goldberg et al. (2004) and Uribarri et al. (2010), who showed that high-fat foods which had high heat treatment, such as French fries, were high in AGE level. Overall, high thermal processing or extended processing time in frying is most likely responsible for producing increased AGE amounts in foodstuffs [2, 19].

The fat-group foods contain high amounts of AGE, while the carbohydrate-group food contains relatively low AGE [19]. Foodstuffs that are high in fat and protein contain higher levels of MGO than foods rich in carbohydrates. Uribarri et al. (2010) observed high AGE concentrations in all foodstuffs, including fat like olive oil, butter, cheese, and milk products [2]. Goldberg et al. (2004) and Uribarri et al. (2010) quantified the amounts of CML in 549 commercially available foodstuffs using ELISA. They revealed that the highest CML amounts were in foods rich in fat (mean as high as 100 ± 19 kU/g), and foods rich in carbohydrate had a relatively low amount in 3.4 ± 1.8 kU/g [2, 19].

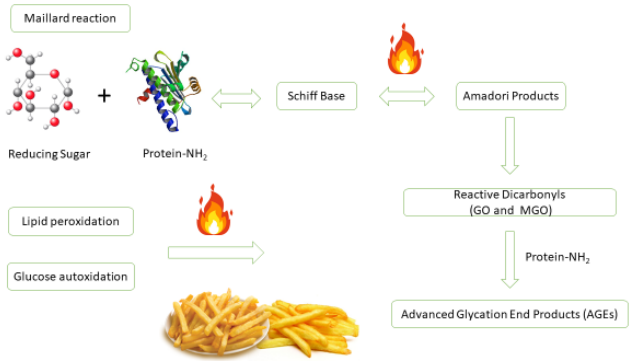

Figure 3 The formation process of AGE precursors in French fries.

Consistent with the findings of the present study, Yamagishi et al. (2007) have listed AGE-rich and poor food items and reported that French fry (white potato) is one of the AGE-rich food items [20].

The preparation of foods under varying water and thermal conditions has a different effect on dietary AGEs level. Uribarri et al., (2010) analyzed the AGE level of foods which were subjected to usual cooking techniques such as deep-frying (180 °C), oven-frying (230 °C), boiling (100 °C), roasting (177 °C), and broiling (225 °C). The AGE concentration of each food was given as AGE kilounits/100 g food. In all food groups, exposure to higher temperatures and lower water contents matched with higher dietary AGE contents for an equal weight of food than foods prepared at lower temperatures or with more water. Accordingly, frying, broiling, grilling, and roasting yield more dietary AGEs than boiling, poaching, stewing, and steaming. As a result of the study, AGE amounts observed to range 1–27,000 kU per serving [2].

Goldberg et al. (2004) investigated the AGEs in commonly consumed foodstuffs and revealed that fat-group foods exhibited the highest level of AGEs. According to their results, the concentration of AGEs existing in all food groups was associated with cooking temperature, duration of cooking, and water existence. Frying (177 °C) and broiling (225 °C) lead to the highest concentrations of AGEs, followed by roasting (177 °C) and boiling (100 °C). The tendency for AGE levels achieved was oven frying > deep frying. In all food groups, exposure to higher temperatures reaches a higher AGE amount for the same weight of food [19].

As seen in Table 1, there is a significant correlation between the fat amount of foods and MGO with dietary AGEs level. In the present study, high-heat-processed food such as French fries had higher levels of MGO than GO. For GO and MGO, these results were in accordance with data reported in the literature [2, 19].

Frying accelerates the formation of new AGEs. Uribarri et al., (2010) [2] reported that uncooked oil and butter showed low levels of MGO, but in dry-heated fat, as in French fries, MGO levels were significantly higher. According to these data, French fries (white potatoes) contain 41 to 90 times higher AGE than boiled white potatoes. Besides, they reported approximately a 2-fold lower AGE amount in homemade French fries as compared to the data of French fries subjected to fast-food cooking.

Food preparation and processing are now well-known to determine dietary AGE formation. Primarily, high temperatures and long cooking times increase the formation of AGE. AGEs are formed during Maillard reactions in food processing. The precursors of AGEs, GO and MGO, are formed by oxidation of proteins and fats in food processing. The formed GO and MGO compounds react with the residues of lysine and arginine in proteins to form the final AGEs. The MGO is very reactive than GO and generates CEL and hydroimidazolones [21]. Recent investigations state a significant correlation between the amount of CML and MGO by different cooking methods in foods. Increasing the cooking temperature increases the amount of CML 200 fold in the same foodstuff. The CML content is used to detect the amount of dietary AGE levels in foods. There is a significant correlation between the fat amount of foods and MGO with dietary AGEs level. Besides, one study revealed high fat ripened cheeses contained a higher level of dietary AGEs than low-fat cheeses such as reduced-fat mozzarella and 2% milk cheddar [2]. Moreover, high fat containing spreads such as butter, mayonnaise, cream cheese, and margarine have high dietary AGEs. The kind of cooking fat used in food preparation causes the formation of an altered amount of dietary AGEs [22]. In the literature, Fujioka and Shibamoto (2004), reported the GO and MGO levels as 0.8-4.0 and 0.2-1.3 mg/kg in oils (cooked) [23], Degen et al. (2012) reported that MGO amounts were 1.8−68 mg/100 g in cookies [24]. Arribas-lorenzo and Morales (2010) were studied the levels of GO and MGO in commercially available cookies and found between 4.8 and 26.0 mg/kg and between 3.7 and 81.4 mg/kg, respectively [25].

Lipid-derived reactive α-dicarbonyl compounds such as GO and MGO are formed by lipid peroxidation [3, 4]. Jiang et al. (2013) compared the total α-dicarbonyl compounds in butter, margarine, safflower oil, beef fat, and cheese after heating at 100 °C and 200 °C. The total α-dicarbonyl levels were increased 55 fold in butter and 15 fold in margarine [26]. The cooking temperature for French fries is between 177 and 230 °C [2]. Mostly, higher cooking temperatures and fat content in foods increase the production of AGE precursors.

Recent investigations revealed a strong relationship between insulin resistance and beta-cell dysfunction with MGO levels [7]. Sandu et al. (2005) have demonstrated that insulin resistance and type 2 diabetes in high-fat-fed mice are related to high glycotoxin intake [27]. Thornalley (2005) [28] found a correlation with increased diabetic complications in animal experiments. When dietary AGEs are given to non-obese animals, type 1 diabetes mellitus develops. It is thought that dietary AGEs disrupt the beta cells in the pancreas. As mentioned above, MGO is a very reactive α-dicarbonyl compound and formed at harmful final AGEs. High consumption of foods with high MGO content can cause an occurrence of many chronic diseases.

Intervention researches investigating the effect of dietary AGEs are mainly depending on diets where the cooking technique was altered. Varied cooking methods affect not only the contents of AGEs in the diet but also other Maillard reaction products such as hydroxymethylfurfural and acrylamide. High-temperature cooking methods have been revealed to cause various changes in diet composition [29]. For instance, high cooking methods reduce the water amount of foodstuffs, so that caloric intake per 100 g foodstuff increases. High temperatures also lead to degradation of

micronutrients, and frying increases fat and total caloric intake.

With the progress in determination methodologies, the amounts of AGEs in many foodstuffs can now be detected, offering valuable data on dietary choices for the population, particularly for people with diabetes. Such data is also beneficial for knowing the formation and inhibition of AGEs during food processing. The first important step to demonstrate whether dietary AGEs pose a risk to human health is to have access to the accurate methodology to measure AGEs and to build up a reliable dietary AGE database. This study presents an accurate HPLC methodology to detect AGE concentration in foodstuffs. The main advantage of the current study is the use of a HPLC system and the ability to examine two major AGEs in one run. To further examine the significance of dietary AGEs in disorders, the presented consistent analytical procedure is proper to investigate the GO and MGO in foods and is a powerful tool for establishing a wide-ranging dietary AGE database.

4. Conclusion

GO and MGO are the main precursors of AGEs. Therefore, in this study, the AGE precursors, GO, and MGO, in French fries were determined. AGEs are formed throughout food processing and storage. The accumulation of these AGEs has harmful effects on the human body. The increased level of AGEs in the circulation is linked with diabetes mellitus and Alzheimer's complications. Also, the amount of AGEs in foods is correlated with increased fat content and cooking temperature. The French fries used in this study contain high fat and cooked at high cooking temperatures. Therefore, these foods had higher quantities of AGE precursors. People who often consume foods rich in AGEs will be at a higher health risk than those who consume less. The health problems related to AGEs can be reduced with an AGE-restricted diet. Therefore, it is suggested to reduce the consumption of foods rich in AGEs to reduce high AGE intake in the diet. More research is needed on AGEs in foods and their reduction in the diet.

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