# Analysis Methods of Common Biological Components in Food Additives

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***Abstract*—The diseases caused by food are defined as the illnesses with which people are infected or threatened by the foods they eat. These diseases are a broad public health issue and cost a lot to individuals and authorities. In this view, food safety should be a big deal for whole world and paid a lot of attention. Analysis of biological components in food additives plays a vital role in the industry since it can detect and quantify all the main components (sugar, nucleic acid, protein and fat) we can find. With the development of technology, a lot of new created approaches such as western blotting, chromatography, RT PCR are applied in this area which shows some advantages over the conventional ones. In addition, the new technology can be further developed in the direction of simplicity and speed in the analysis work. In this review, we attempt to focus on the approaches on the analysis of different biological components and summarize them in a whole glance.**

***Index Terms*—food, biological components, fat, protein**

## I. Introduction

It is common sense that food safety has become a vital issue challenging worldwide and drawing big attention. The important factors for food safety contain environment and products. Food additives play an important role in the food industry and are broadly used for keeping food quality and characteristics. Right now, food additives in food have got much attention due to their potential side effects on human beings. During the past years, the common use of food additives has been the most obvious issue for a long time. Hence, additives have become routine ways [1]. The most common additives that have broadly been found in food are nitrogen components, which can be mixed with protein, sugar, preservatives, and metabolites. Due to the additives existing, multiple detection methods should be applied in this area since sometimes the additives can affect the human health [2]. For example, sugar is an important component in food as an additive, while excessive use of sugar can lead to diabetes and other serious health problems. So, it is very important to control the usage of sugar in which sugar detection and right labelling for the food will be a good way. Several approaches can be used to detect sugar, and other food biological components. It shows great significance to summarize all the existing well-established methods for all the biological components analysis [3]. In this article we expand the discussion on the current detection methods of common nutrients in food and their advantages and disadvantages, and hopefully it can shed light on the approach's development.

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## II. Protein Components Analysis

In order to speed up food processing and improve food quality, people often use the biocatalytic effect of protease additives to achieve this purpose. Western Blot (WB) is an important laboratory technique that can specifically identify and characterize proteins [4]. In the Fig. 1(a), WB detects proteins, "probes" are antibodies, and "colors" use labeled secondary antibodies. This method uses Polyacrylamide Gel Electrophoresis (PAEG) to separate complex proteins of different charges and molecular weights, and selectively detects them using an antibody-mediated reporter system. After separation, proteins are transferred to a solid carrier as shown in Fig. 1(b, c), such as a nitrocellulose membrane, which adsorbs proteins in the form of non-covalent bonds and can keep the types of peptides separated by electrophoresis and their biological activities unchanged. Proteins on the solid carrier are used as antigens to react with the corresponding antibody, and then a specific type of enzyme or isotope-labeled secondary antibody is added to form a special structure of protein-antibody-enzyme-labeled antibody [5]. Substrate color or development can show that the protein-antibody-enzyme-labeled antibody of a specific structure exists in the form of a "band" [6]. This approach is often used to detect some specific protein in a biological sample and give information on its molecular size.

The first step is preparation of sample that usually makes the protein to be dissolved in the lysis buffer, generally including detergents and protease inhibitors. These soluble protein samples are diluted with a concentrate of loading buffer containing indicator dyes such as bromophenol blue, sodium dodecyl sulfate (SDS) and glycerin. Then these loading proteins shall be heated between 70°C and 100°C for 5-10 minutes. This process will cause the loss of the protein's secondary conformation structure, and negative electrons will wrap these proteins so that the protein migrates towards the positive electrode after the current is applied. The proportion of polyacrylamide in the gel and buffer will affect the movement of the protein in the gel [7]. The protein is

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separated by the gel and transferred and fixed on the nitrocellulose or Polyvinylidene Fluoride (PVDF) membrane. The best separation and resolution of the target protein of the expected size can be achieved by selecting the best gel/buffer system. After the membrane is cleaned by the specific primary antibody, it is blocked and incubated for a period of time, and then a special labeled secondary antibody that can bind to the primary antibody is added. Finally, the expression of the protein was obtained by analyzing the position and depth of the coloring (Fig. 2) [8].

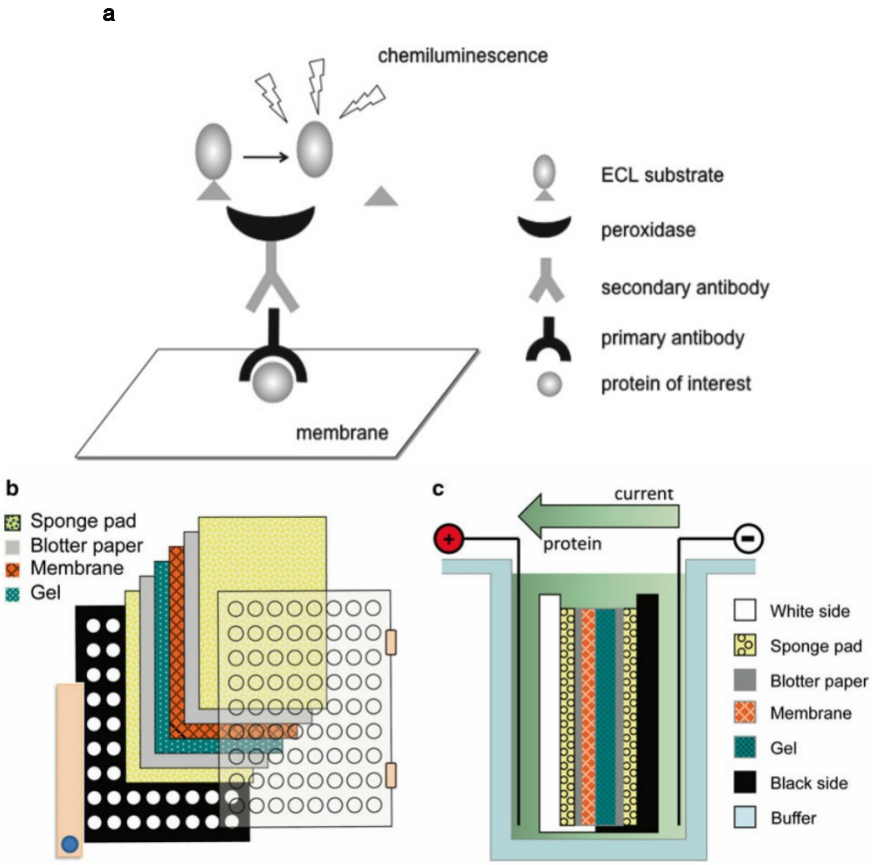


Figure 1. (a) schematic presentation for the detection of proteins on the western blot membrane by ECL [9]. (b) Schematic diagram of the position of components in a protein transport cassette. (c) The composition of the components in the protein transfer tank and the orientation of the current flow [5].

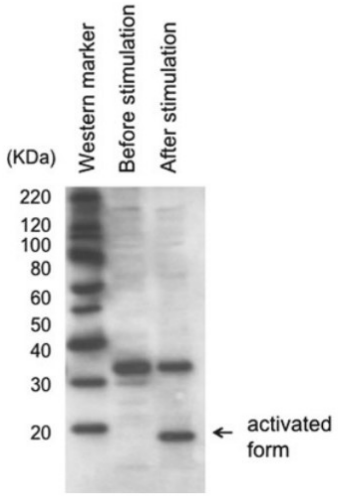


Figure 2. A typical western blotting image after being exposed to the wrapped membrane in a film cartridge, the X-ray film is developed in a dark room [9].

## III. Nucleotide Compomponents Analysis

### A. Normal Polymerase Chain Reaction (PCR)

Nucleic acid food additives are generally used in condiments such as monosodium glutamate, chicken essence, and soy sauce to improve freshness. Adding a little to the food can significantly enhance the taste and improve the original umami and aroma of the food. At present, the commonly used method for identifying and detecting nucleic acid is PCR. The principle of PCR technology seems like the natural replication of DNA. With the assistance of DNA polymerase, DNA will replicate into the same two strands based on the principle of base complementary pairing. Molecular copy. Therefore, using temperature changes controlling the denaturation and renaturation of DNA, with designed primers, dNTPs and DNA polymerase can finish the in vitro replication of specific genes [10]. The ladder curve usually appears during PCR amplification, like the reverse transcriptase or virus in the Fig. 3.

PCR specificity depends on oligonucleotide primers which are complementary to two ends of the target sequence. PCR usually contains two sequential amplification reactions, each one relying on a different paired primer. Products of the first amplification reaction will be treated as the template for the second round PCR, initiated by the oligonucleotides placed inside the first pair of primers. Using two pairs of oligonucleotides allows more cycles to be run as a result to improve the sensitivity of PCR [11].

The PCR reaction has three main steps: denaturation, annealing and extension. Repeat these steps using an automatic thermal cycler until sufficient amplification has been performed (usually >25 cycles). ① DNA denaturation: At 93°C, after heating the DNA for a certain time, the template DNA double-strand is dissociated. ② Annealing: Annealing is carried out at a lower temperature, usually between 45 and 60°C, and primers are paired with the complementary sequence of the template DNA single strand; ③ Extension: at 72°C, under the action of DNA polymerase, dNTP is used as the reaction raw material, the target sequence is the template, and according to the principle of base complementary paired and semi-reserved replication, and then a novel semi-reserved replication strand is synthesized [10].

Factors affecting PCR:

① Strictness of annealing step: Increasing the annealing temperature can reduce mismatched hybridization. ② Shortening the annealing time and extension time can reduce error initiation and error extension. ③ Primer dimers are the most common by-products. Lowering the concentration of primers and enzymes can also reduce false triggers, especially primer dimerization. Increase the temperature is also another method to make it. ④ Changing the concentration of $MgCl_2$ can increase the severity of the reaction or have a direct effect on Taq enzyme.

Compared with the traditional PCR method, RTPCR has different principles, requirements, and results: RTPCR is monitored in real time by fluorescence excited by a fluorescent dye bound to DNA, while ordinary PCR is to detect the fluorescent dye inserted in the DNA to see if there is any Destination fragment. At the same time, the requirements are also different. Fluorescence quantification has higher requirements for amplified fragments, generally 100-300bp. And RTPCR can quantitatively analyze the product without electrophoresis. In the final analysis of the results, RTPCR can achieve precise quantification, but ordinary PCR cannot. RTPCR quantification is reflected in the ability to show the whole amplification process, amplification curve, dissolution temperature and melt curve, which cannot be achieved by regular PCR [12], [13].

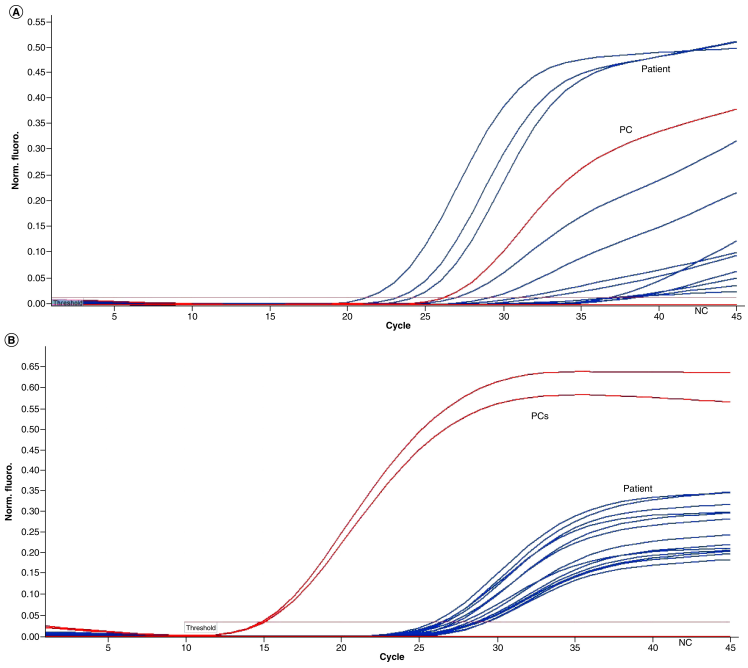


Figure 3. (A) Reverse transcriptase-PCR amplification yield results. (B) in Rotor-Gene Q Software 2.3.1. NC: Negative control; PC: Positive control; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2 [15].

### B. Real-Time Quantitative PCR (RT-QPCR)

RT-QPCR is similar to PCR in primer design, but there are some differences. Their similarities are as follows: 1. The sequence search is consistent, and both select the conserved segment of the target gene 2. Select the appropriate amplified fragment Size 3. Avoid the formation of 4 or more consecutive pairings and circular hairpin structures between the primer itself or with the primer; 4. Avoid the TM difference between the primers exceeding 2℃; 5. Avoid the 3'end of the primer 3 One or more consecutive identical bases; on this basis, the primer design of RT-QPCR is more stringent: 1. The length of RT-QPCR primers should be within 300bp, generally between 80-150bp is preferred; 2. Multiple pairs of target genes are simultaneously expanded To increase, it is necessary to design primers that are as consistent as possible; 3. When the target gene content is relatively low, it is necessary to design primers with higher sensitivity; 4. RT-QPCR requires less primer dimers and high requirements for the secondary structure of the primers [14].

## IV. Fat Components Analysis

Fat is an important component of food additives, and it is also one of the main components of the human body. Common fat additives such as preservatives, emulsifiers, can increase the preservation time of food and improve the flavor of food. But fats can be hydrolyzed to produce fatty acids and glycerol. Fatty acids can be divided into saturated fatty acids and unsaturated fatty acids according to the presence or absence of unsaturated double bonds. Some of the unsaturated fatty acids are trans fatty acids, which can cause coronary heart disease and other cardiovascular and cerebrovascular diseases. At present, there are many methods for determination of fatty acids, including titration, copper soap colorimetry, chromophore spectrophotometry, chromatography, etc. Jensen [16], [17] reported two simple and convenient methods for detecting fatty acids, the titration method and the copper soap colorimetric method. Of the two methods, the copper soap colorimetric method is better than the titration method. The copper soap colorimetric method was originally invented by Duncombe [18]. It mainly uses lipase to hydrolyze olive oil, tributyrin and triolein to produce fatty acids and glycerin, and copper ions in fatty acids and color developers. When ph=6, the reaction produces a copper soap blue chromium compound. The absorption value of this complex can be calculated by a spectrophotometer, and then the fatty acid content can be obtained by comparing the standard curve. However, this method is relatively complicated, and at the same time, due to the interference of metal ions, it will also affect the accuracy of the detection.

At present, the most commonly used method for detecting fatty acids is chromatography. Chromatography is also called chromatography or chromatographic analysis. This method can perform qualitative and quantitative analysis and separation. The working principle of chromatography is to separate different substances according to the different selective distributions of different phases. When the mobile phase drives the sample of the mixture to be tested into the stationary phase, the distribution ratio of the different components in the sample to be tested is different. Different speeds move and elute along the stationary phase, and finally achieve the purpose of separation and analysis. Due to the characteristics of its principle, different component peaks will appear in the chromatogram (Fig. 4).

Chromatography can be divided into gas chromatography and liquid chromatography according to different mobile phases. Due to the rapid transfer rate of the sample in the gas phase, the separation efficiency of the gas phase is very high, but the gas phase has greater limitations in the analysis of high-boiling fatty acids that are easy to decompose [19]. Liquid chromatography is not affected by the volatility and thermal stability of the sample, so it is widely used in fatty acid detection [20].

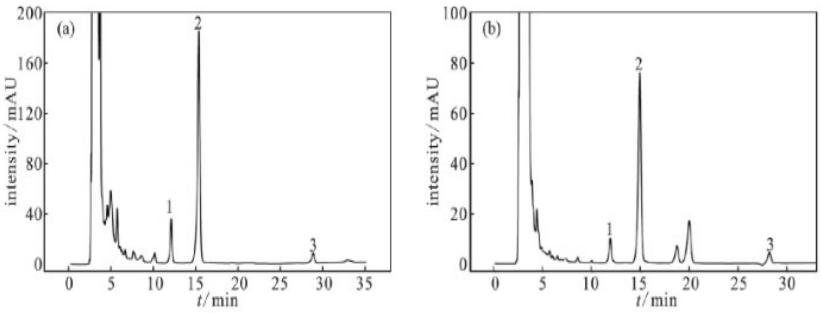


Figure 4. (a) HPLC spectra of standards(α-linolenic acid: linoleic acid: stearic acid = 1: 1: 5) (b) and fatty oil from Urtica cannabina L.seed14 1. Alpha-linolenic acid 2. Linoleic acid 3. Stearic acid.

## V. Carbohydrates Components Analysis

Carbohydrates are an important class of organic compounds in food additives. For example, cyclamate is a sweetener, which is 40 times sweeter than sucrose; and sorbitol, which can enhance the flavor of food and maintain the function of food aroma. Carbohydrates is an organic compound of polyhydroxy aldehydes and polyhydroxy ketones. It can be divided into monosaccharides, disaccharides, polysaccharides and complex sugars. Monosaccharides and polysaccharides can be distinguished by Buff's reagent, which is a weakly

acidic solution composed of 5% copper acetate and 1% dilute acetic acid solution. Under acidic conditions, the reduction rates of monosaccharides and polysaccharides are significantly different. Monosaccharides can reduce copper ions within 2 minutes to produce brick red cuprous oxide, resulting in orange or orange precipitation, while the reduction rate of polysaccharides is slow many. According to the structure of sugars, it can be divided into aldose and ketose. The former has aldehyde group and the latter has carbonyl group. Typical examples are glucose and fructose. The identification of ketose can be done through the Seliwanoff experiment. An equal volume of concentrated hydrochloric acid and a few drops of Seliwanoff reagent are added to a sample dissolved in water, and the resulting mixture is heated just to boiling. If the solution appears red within 2 minutes, and dark black precipitates are formed, it indicates the presence of ketose [21].

The detection of carbohydrates generally has problems of many types, low content, complex sample matrix, and a large number of isomers and chiral compounds, which brings great challenges to the detection of carbohydrates. There are many methods for determining sugars, including volumetric method, polarimetry, colorimetry, chromatography and refractometry. These methods have their own advantages, but they are all cumbersome. In recent years, chromatography has been widely used in the determination of sugars. Because of its good separation ability, glucose can be separated from reducing sugars such as fructose, lactose, and maltose, which greatly facilitates the detection of soluble sugars. Chromatography includes gas chromatography, liquid chromatography, ion chromatography, etc. At first, thin-layer chromatography was used to separate various carbohydrates, but this method can only be used for qualitative purposes, and the quantitative accuracy is very poor. Gas chromatography has the advantages of higher sensitivity, better separation effect, simple operation, etc. However, this method has certain requirements on the boiling point and stability of the substance. Before the determination by gas chromatography, the extract must be derivatized Treatment makes the less volatile sugar substances (such as glucose fructose) converted into more volatile substances [15]. The process of this method is more complicated, it takes a long time, and it also causes a lot of trouble for the quantification of carbohydrates, so it is not widely used at present. High performance liquid chromatography (HPLC) does not require too much sample, as long as it can be made into a solution, it can be measured (Fig. 5). Therefore, materials with poor thermal stability and higher boiling points can be measured and analyzed by this method. The detector uses a refractive index detector (RID). Although the sensitivity is poor, it can quickly detect free small-molecule sugars [16]. It has been widely used in sugar determination in recent years. Ion chromatography is a new type of analytical technology that has recently emerged. It uses a high-efficiency anion exchange column and a pulsed amperometric detector to analyze samples. This method is more sensitive, has better selectivity, requires less samples, and has strong automation capabilities. However, it has a major disadvantage that the analysis requires more time [17].

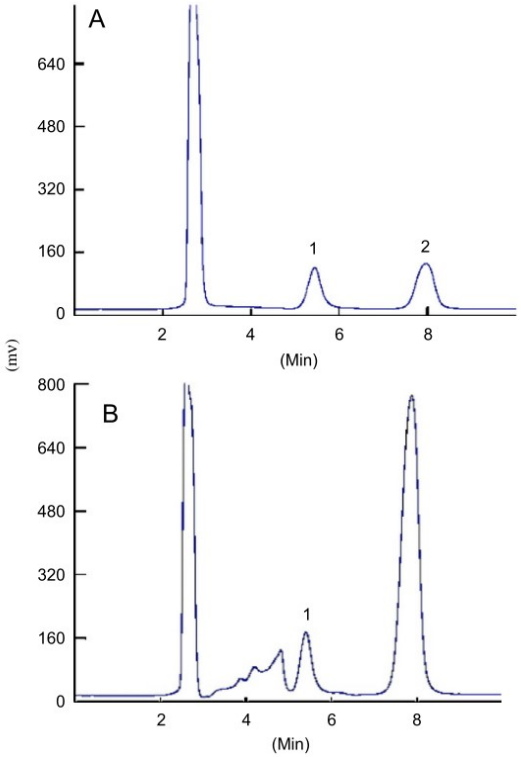


Figure 5. Typical chromatograms of saccharide analysis by the HPLC method. Saccharide peaks: (A) Saccharide standards and (B) milk-based formulae sample [18]. 1, glucosamine; 2, lactose.

## VI. Conclusion

To conclude, the article reviewed in this paper indicates that several analytical methodologies have been created to monitor and detect the main food biological components like protein, fat and sugars. Although diverse methods are used in the food industry, including conventional and creative techniques, it still shows high demand for that novel, rapid and efficient food fingerprinting methodologies to be adopted by both inspection authorities and industry to evaluate the food and evaluate their authenticity. The challenges in the possible applications of food detection approaches in the official food control include the establishment of harmonized operating protocols, the standardization of both chemical analysis and natural components evaluation.

## Conflict of Interest

The author declares no conflict of interest.

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## References

[1] F. C. O. L. Martins, M. A. Sentanin, D. D. Souza, "Analytical methods in food additives determination: Compounds with functional applications," *Food Chemistry*, vol. 272, pp. 732-750, 2019.
[2] K. S. Rowe and D. R. Briggs, "Food additives and behaviour," *Med. J. Australia*, vol. 161, pp. 581-582, Aug. 1994.
[3] P. D. Silva, R. Cruz, and S. Casal, "Sugars and artificial sweeteners in soft drinks: A decade of evolution in Portugal," *Food Control*, vol. 120, p. 107481, 2021.
[4] B. Kim, "Western blot techniques," *Methods Mol. Biol.*, vol. 1606, pp. 133-139, 2017.
[5] T. S. Hnasko and R. M. Hnasko, "The western blot," *Methods Mol. Biol.*, vol. 1318, pp. 87-96, 2015.
[6] S. C. Taylor and A. Posch, "The design of a quantitative western blot experiment," *Biomed. Res. Int.*, vol. 2014, p. 361590, 2014.
[7] R. Ghosh, J. E. Gilda, and A. V. Gomes, "The necessity of and strategies for improving confidence in the accuracy of western blots," *Expert Review of Proteomics*, vol. 11, pp. 549-560, 2014.
[8] M. Mishra, S. Tiwari, and A. V. Gomes, "Protein purification and analysis: Next generation western blotting techniques," *Expert Review of Proteomics*, vol. 14, pp. 1037-1053, 2017.
[9] S. Hirano, "Western blot analysis," *Methods Mol. Biol.*, vol. 926, pp. 87-97, 2012.
[10] K. Canene-Adams, "General PCR," *Methods in Enzymology*, vol 529, pp. 291-298, 2013.
[11] M. R. Green and J. Sambrook, "Nested Polymerase Chain Reaction (PCR)," in *Cold Spring Harbor Protocols*, Cold Spring Harbor Laboratory Press, 2019.
[12] A. Anantharajah, R. Helaers, J. P. Defour, *et al.*, "How to choose the right real-time RT-PCR primer sets for the SARS-CoV-2 genome detection?" *J. Virol. Methods*, vol. 295, p. 114197, 2021.
[13] C. A. Heid, J. Stevens, K. J. Livak, *et al.*, "Real time quantitative PCR," *Genome Research*, vol. 6, no. 10, pp. 986-994, 1996.
[14] B. Thornton and C. Basu, "Rapid and simple method of qPCR primer design," *Methods Mol. Biol.*, vol. 1275, pp. 173-179, 2015.
[15] F. Sarigul, O. Doluca, S. Akhan, *et al.*, "Investigation of compatibility of severe acute respiratory syndrome coronavirus 2 reverse transcriptase-PCR kits containing different gene targets during coronavirus disease 2019 pandemic," *Future Virol.*, vol. 15, pp. 515-524, 2020.
[16] F. Q. Nuttall and D. S. Wedin, "A simple rapid colorimetric method for determination of creatine kinase activity," *J. Lab. Clin. Med.*, vol. 68, pp. 324-332, 1966.
[17] R. G. Jensen, "Detection and determination of lipase (acylglycerol hydrolase) activity from various sources," *Lipids*, vol. 18, pp. 650-657, 1983.
[18] W. G. Duncombe, "The colorimetric micro-determination of long-chain fatty acids," *Biochem. J.*, vol. 88, 7-10, 1963.
[19] K. Takahashi and N. Goto-Yamamoto, "Simple method for the simultaneous quantification of medium-chain fatty acids and ethyl hexanoate in alcoholic beverages by gas chromatography-flame ionization detector: Development of a direct injection method," *J. Chromatogr. A*, vol. 1218, pp. 7850-7856, 2011.
[20] H. Zhao, Y. Zhang, X. Xue, *et al.*, "Analysis of nine antioxidants in vegetable oils by high performance liquid chromatography," *Se Pu = Chinese Journal of Chromatography*, vol. 38, no. 7, pp. 841-846, 2020.
[21] M. Shahidullah and S. S. Khorasani, "The sensitivity and selectivity of the Seliwanoff test for fructose," *Anal. Chim. Acta*, vol. 61, pp. 317-319, 1972.



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