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DETERMINATION, EVALUATION, AND ADDITIVE DETECTION OF IMPORTED WHEAT FLOUR AND COMPARISON WITH PRODUCED LOCAL FLOUR

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BY

Abdalqadir Muayad Abdaljabar AL KUBAISI

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DETERMINATION, EVALUATION, AND ADDITIVE DETECTION OF IMPORTED WHEAT FLOUR AND COMPARISON WITH PRODUCED LOCAL FLOUR

By Abdalqadır Muayad Abdaljabar Al KUBAISI

September 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

DETERMINATION, EVALUATION, AND ADDITIVE DETECTION OF IMPORTED WHEAT FLOUR AND COMPARISON WITH PRODUCED LOCAL FLOUR

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The study was conducted on March 27, 2022, to examine and evaluate 12 types of imported flour from several countries and compare it with local flour (from governmental mills) in Anbar province, Iraq. The findings revealed that the F8 type had the highest average score (91.40), while the F11 type recorded the lowest average score (85.37). The results also indicated significant differences between the types in terms of protein content, where the F7 type had the highest average score (14.06) and the F5 type had the lowest average protein content (11.23). As for the moisture content, the results indicated significant differences between the types, where the F1, F7, and F10 types had the highest average scores (12.83, 12.96, and 13.06, respectively) and the F6 type had the lowest average score (10.96). The F5 type had the highest average score (99.83) in terms of flour permeability through the sieve (8XX), while the F11 type recorded the lowest level of permeability (66.46). The F11 type had the highest average ash content (1.80), while the F8 type had the lowest average score (0.58). The results of the examination of additives and bleaching agents showed the presence of the compound (Azodi Carbon Amide) in types F3, F5, F6, and F7. Additionally, types F2, F4, F8, F9, and F10 were found to contain the compound (Benzoyl Peroxid). The compound (Potassium Bromate) was found in type F1, while the compound (Calcium Peroxide) was found in type F12. The study revealed significant differences in sensory evaluation attributes of the produced loaf bread. The results of the analysis showed that the F7 treatment had the highest mean score (9.80) for crust color, while the lowest mean score (7.70) was recorded for the F6 treatment.

No significant differences were recorded for crumb texture uniformity among the treatments. However, there were significant differences in crumb tenderness between the different types of bread. F1 treatment had the highest mean score (9.50), while F9 treatment had the lowest mean score (8.20). For crumb color, the analysis indicated clear and significant differences among the treatments. F6 and F7 treatments had the highest mean score (9.60), while F3 and F9 treatments had the lowest mean score (8.30). Regarding crumb structure, the F10 treatment had the highest mean score (9.20), while the F4 treatment had the lowest mean score (7.80). The results of the taste and flavor examination showed that the F7 treatment had the highest mean score (18.80), while the F4 and F5 treatments had the lowest mean score (16.70 and 17, respectively).

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Keywords: Flour Quality, Additives, Bleaching Agents, Loaf Bread, Flour composition

ÖZET

İTHAL BUĞDAY UNUNUN BELİRLENMESİ, DEĞERLENDİRİLMESİ, KATKI MADDESİ TESPİTİ VE ÜRETİLEN YERLİ UNLA KARŞILAŞTIRILMASI

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Çalışma, çeşitli ülkelerden ithal edilen 12 çeşit unu incelemek ve değerlendirmek ve Irak'ın Anbar vilayetindeki yerel unlarla (devlet değirmenlerinden) karşılaştırmak amacıyla 27 Mart 2022 tarihinde gerçekleştirilmiştir. Bulgular, F8 tipinin en yüksek ortalama puana (91.40) sahip olduğunu, F11 tipinin ise en düşük ortalama puanı (85.37) kaydettiğini ortaya koymuştur. Sonuçlar aynı zamanda protein içeriği açısından da tipler arasında önemli farklılıklar olduğunu göstermiştir; F7 tipi en yüksek ortalama puana (14.06) sahipken, F5 tipi en düşük ortalama protein içeriğine (11.23) sahip olmuştur. Nem içeriğine gelince, sonuçlar F1, F7 ve F10 tiplerinin en yüksek ortalama puanlara (sırasıyla 12.83, 12.96 ve 13.06) ve F6 tipinin en düşük ortalama puana (10.96) sahip olduğu tipler arasında önemli farklılıklar olduğunu göstermiştir. Elekten un geçirgenliği (8XX) açısından F5 tipi en yüksek ortalama puana (99.83) sahipken, F11 tipi en düşük geçirgenlik düzeyini (66.46) kaydetmiştir. F11 tipi en yüksek ortalama kül içeriğine (1.80) sahipken, F8 tipi en düşük ortalama puana (0.58) sahip olmuştur. Katkı maddeleri ve ağartma maddelerinin incelenmesinin sonuçları, F3, F5, F6 ve F7 tiplerinde (Azodi Karbon Amid) bileşiğinin varlığını göstermiştir. Ayrıca, F2, F4, F8, F9 ve F10 tiplerinin (Benzoil Peroksid) bileşiğini içerdiği tespit edilmiştir. Bileşik (Potasyum Bromat) F1 tipinde bulunurken, (Kalsiyum Peroksit) bileşiği F12 tipinde bulunmuştur. Çalışma, üretilen somun ekmeklerin duyusal değerlendirme özelliklerinde önemli farklılıklar olduğunu ortaya koymuştur. Analiz sonuçları, F7 uygulamasının kabuk rengi için en yüksek ortalama puana (9.80) sahip olduğunu gösterirken, en düşük ortalama puan (7.70) F6 uygulaması için kaydedilmiştir. Uygulamalar arasında iç doku homojenliği açısından önemli bir farklılık kaydedilmemiştir. Bununla birlikte, farklı ekmek türleri arasında

ekmek içi hassasiyeti açısından önemli farklılıklar söz konusu olmuştur. F1 uygulaması en yüksek ortalama puana (9.50) sahipken, F9 uygulaması en düşük ortalama puana (8.20) sahip olmuştur. Kırıntı rengi için analiz, uygulamalar arasında açık ve önemli farklılıklar olduğunu göstermiştir. F6 ve F7 uygulamaları en yüksek ortalama puana (9.60) sahipken, F3 ve F9 uygulamaları en düşük ortalama puana (8.30) sahip olmuştur. Kırıntı yapısı ile ilgili olarak, F10 uygulaması en yüksek ortalama puana (9,20) sahipken, F4 uygulaması en düşük ortalama puana (7,80) sahip olmuştur. Tat ve lezzet incelemesinin sonuçları, F7 muamelesinin en yüksek ortalama puana (18,80) sahip olduğunu, F4 ve F5 uygulamalarının ise en düşük ortalama puana (sırasıyla 16,70 ve 17) sahip olduğunu göstermiştir.

2023, 87 sayfa

Anahtar Kelimeler: Un Kalitesi, Katkı Maddeleri, Ağartma Maddeleri, Somun Ekmek, Un Bileşimi

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Abdalqadır Muayad Abdaljabar AL KUBAISI
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CONTENTS

LIST OF SYMBOLS

'	Minute
"	Second
%	Percentage
°	Degree
mg	Milligram
gr	Gram
ha	Hectare
kg	Kilogram
L	Loam
lt	Liter
Mol	Mol
ºC	Celsius
ppm	Parts per million
Xx8	Sieve opening size
$P<0.05$	Statistical probability of the analysis results
M	Molarity
r=	Variable value

LIST OF ABBREVIATIONS

ADA	Azodicarbonamide
CRD	Complete Randomized Design
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization
GRAS	Generally Recognized as Safe
HPLC	High-Performance Liquid Chromatography
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KBrO3	Potassium Bromate
NACL	Sodium chloride
PVC	Vinyl Chloride
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SE	Size Exclusion
SEM	Scanning Electron Microscopy
SKCS	Single-kernel characteristics system
WHO	World Health Organization

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1. INTRODUCTION

Cereals are the mainstay of human food worldwide, especially wheat, as its products provide a rich source of energy and protein necessary for the general population (Zilic 2013), where wheat occupies the first place in terms of the cultivated area and the third place in terms of the amount of production (FAO 2016). In addition to containing protein, wheat also contains carbohydrates, fiber, fats, amino acids, and vitamins (Elmalimalid 2018).

Wheat flour is one of the most important nutrients in the world, and it is considered a basic food item. Humans depend on it for most of their food, as it has been used in the manufacture of bread and pastries since ancient times, as it is the main source of energy and some nutrients such as vitamins, fats, and proteins. That's because wheat contains a high percentage of proteins, which increase the percentage of amino acids that provide the human body with energy. It also contains enzymatic or chemical endogenous redox and oxidative elements that affect the gluten reticulum. If it is missing, it will be added from external sources (Joye *et al.* 2009). The functional properties of a substance like flour depend on gluten, which focuses its attributes on items such as wheat and different field climates such as adding fertilizer and weather conditions, watering, and then harvest and storage. Infection by fungi and insects affects gluten, which directly affects the quality of dough (Ilrik *et al.* 2003; Torbica *et al.* 2007; Georgette *et al.* 2008). that led to adding some materials and improvers for flour that improve product characteristics (Amemiya and Menjivar 1992, Armero and Collar 1998). It features bread and pastries made of flour. Wheat contains a low percentage of antioxidants, so its efficacy is low. Since ancient times, bread has been made from grains and water, and a variety of other ingredients have been added to make it more palatable or to help preserve it. With the passage of time, some ingredients were discovered to improve the other properties of bread and make it safer to eat. Salt, vinegar, and sugar are traditional methods of preservation, and adding a little fat to a recipe makes the product softer to eat and helps keep it unstaining (Gunenc *et al.* 2013).

Wheat varieties differ in their characteristics and uses. Soft wheat is used for the purpose of making pastries, such as cakes, biscuits and bread, while hard wheat (Durum) is used used for the production of pasta and bulgur as it is characterized by its hardness and high content of gluten, which is higher than bread wheat. Wheat contains many important nutrients and minerals such as zinc, iron, magnesium, manganese, vitamins such as B12 and antioxidants. It is also a rich source of fiber (Magallanes-Lopez *et al.* 2017).

Wheat and its derivatives are among the most important natural materials that humans have fed on since ancient times (6000 BC). (Arranz-Otaegui *et al.* 2018) The Romans were the first to grind grain in conical mills. In the beginning of the Industrial Age, in 1930, the first steam mill was built in London. The world’s production of wheat annually has reached more than 789.7 million metric tons (Beef 2023). Among the countries that produce the most are China, India, Russia, and the United States, where China ranks first in production, as these countries produce about a quarter of the world’s production of wheat, according to information provided by the Organization of Food and Agriculture that belongs to the United Nations' statistical database, and the information extracted from the figures of the International Grains Council showed that China's production for the year 2017 amounted to 134.3 million tons, and in 2018 it amounted to 131.4 million tons. Metric studies have also shown that Asian countries are the world's largest importers and consumers of wheat (Egypt, Indonesia, Turkey, China, and Algeria).

The additives added to wheat, such as bleaching agents, preservatives, and chemicals, are used in the manufacturing process to improve its quality and make it more visually appealing. Preservatives are also added to prevent spoilage and increase its shelf life, and they are generally considered safe. There are also chemical additives used in wheat production that improve its appearance, taste, and shelf life. Although these additives are generally safe, consuming them in large quantities can be harmful to human health and can cause diseases such as allergies and digestive system diseases, as well as increase the risk of cancer and heart disease.

In light of the difficult conditions that Iraq has gone through during the past two decades of wars and problems that affected the industrial field, which led to the dependence of the market by a large percentage on flour imported from different countries, and as a result of consumption increases and the demand for wheat flour, a large gap between consumption and global production has shown many health problems among flour consumers because of low-quality manufacturing processes in addition to industrial fraud in adding chemicals that improve the manufacturing properties of bread and pastries, but at the same time, it negatively affects human health. Hence, the study aimed to:

1. Examine some varieties of imported flour in Iraq and available in the local markets to identify the bleaching agents, additives, and fortifying substances added during the manufacturing process and their concentrations.

2. Assess the impact of the chemical additives in flour on the quality of baked goods produced from different flour varieties and compare them to local flour.

3. Sensory evaluation of the bread produced from various imported flour varieties and compare them to locally produced flour without additives.

2. LITERATURE REVIEW

2.1 Wheat

Wheat is considered the most important field cereal crop, belonging to the species Aestivum and the genus Triticum. The grains belong to the grass family (Poaceae), where wheat occupies the first rank in terms of cultivated area and the third rank in terms of global production quantity (Uppal and Sriniras 2004). Wheat is one of the most widely spread crops in the world in terms of production and consumption. It is the main source of energy, proteins, and fibers for human food and animal feed. In addition to proteins, wheat contains carbohydrates, fats, amino acids, dietary fibers, minerals, water, and vitamins (Zilic 2013, Elmalimadi 2018). Wheat is the main global commodity, with an annual production of around 600 million tons (Jasem 2018). Wheat grows on vast areas more than any other food crop, reaching 220.4 million hectares until 2014. The globe is constantly cultivating and producing the crop throughout the year. In Iraq alone, the wheat-cultivated area reached around 7 million dunams in the years 2016–2017, with a production rate of 2,177,885 tons, which is equivalent to 690.5 tons per dunam (Central Statistical Organization Iraq 2018).

The cultivable area in Iraq is estimated to be around 48 million dunams, which represents less than 15% of the total area of Iraq (Jasem 2018). Tudies have indicated that wheat cultivation began to expand from the Fertile Crescent region (an agricultural region in the Middle East that extends in a crescent shape from the Mediterranean Sea to the Tigris and Euphrates rivers and down to the Arabian Gulf) around 8000 BC. Genetic analysis of wild wheat species such as Triticum boeoticum suggests that the first growth occurred in the mountains of southeast Turkey (Mount Karadağ) and nearby regions in Syria and Iraq. Today, there are many hybrid varieties of wheat that have been produced using various hybridization and genetic manipulation techniques. (Central Statistical Organization Iraq 2018).

2.2 Effect of Wheat Type on the Type of Flour Produced.

The type of wheat used in the process affects the type of flour produced. Different types of wheat, such as hard wheat, soft wheat, red wheat, and white wheat, can be used in flour production. The type of flour produced varies depending on the type of wheat used, as well as the milling and manufacturing process, and can have different flavors, textures, and properties. Additionally, different types of flour are used in the production of different products, such as bread, pastries, and sweets, and the appropriate type of flour is chosen based on the type of product being produced (Finney *et al.* 1987).

Bakery products can be classified into several categories based on the type of wheat used in producing the flour. These categories include:

Bakery products made from white refined flour: These products are made from flour that has had its main nutrients removed, such as fiber and other nutrients. This type of flour is used in producing white bread and light pastries.

Bakery products made from whole wheat flour: These products are made from flour that contains the complete nutrients of the grain, including fiber, vitamins, and minerals. This type of flour is used in producing brown bread and heavy pastries.

Bakery products made from medium flour: These products are made from flour that contains moderate amounts of nutrients and fiber. This type of flour is used in producing medium bread and heavy pastries.

The appropriate type of flour is chosen according to the desired product and the nutritional requirements of consumers (Kent and Evers 1994).

Different varieties of wheat are mixed to improve the properties of the resulting flour and obtain baked products of better quality. For example, different wheat varieties can be mixed to improve the milling properties, such as protein and moisture ratios, and thus the quality and baking properties of the flour. Different wheat varieties can also be mixed to enhance the sensory properties of baked products, such as flavor, color, and texture. In general, the mixing of different wheat varieties aims to achieve an ideal balance between the physical, chemical, and sensory properties of the flour and the baked products produced from it (Schiller 1984).

2.3 The Chemical Composition of Wheat

Wheat flour contains a variety of chemical compounds, including protein, fat, carbohydrates, fiber, ash, vitamins, and minerals. The proportion of these components varies depending on the type of wheat, growth and harvesting conditions, processing methods, and storage. Typically, wheat flour contains about 11–13% protein, 1-2% fat, 70–75% carbohydrates, 1.5–2.5% fiber, and 1.5–2% ash, in addition to some vitamins such as B1, B2, B3, and E and many minerals such as iron, calcium, zinc, and magnesium (Kowalski *et al.* 2022).

2.4 Qualitative Characteristics of Wheat Flour

Wheat flour is a basic food ingredient that is commonly used in the production of baked goods, pastries, and pasta. The qualitative characteristics of wheat flour depend on several factors, such as the type of wheat, its source, the method of grinding, and storage. Some of the important qualitative characteristics of wheat flour include:

2.4.1 Flour color

The color of the produced wheat can vary depending on the type of wheat and the processing method. Generally, wheat is characterized by a yellow to light brown color, and the color of wheat can be affected by factors such as soil, weather, and agricultural practices.

The color characteristic of wheat flour refers to the color resulting from the quantity and quality of the brown spots in the flour, which largely depends on the type of wheat and its processing. The color of the flour is very important in food industries that use flour as a raw material, as it greatly affects the final color of the end product. The color of wheat flour ranges from pure white to dark brown, and the flour is usually classified according to its extraction rate or other physical characteristics such as grain size, gluten content, ash content, protein content, and mineral content. It is important to note that artificial dyes may be added to improve the color of the flour, which can affect the quality and health of the flour (Zhai *et al.* 2018).

The impact of wheat flour color can be seen in several ways, including:

Nutritional content: The dark color of wheat flour may indicate higher levels of nutrients such as fiber, vitamins, minerals, and amino acids. Conversely, the lighter color may indicate lower levels of these nutrients.

Baking performance: The color of wheat flour can affect its baking performance. Darker-colored flour contains higher levels of proteins and gluten, which can increase its baking performance.

Taste and aroma: Wheat flour color can affect the taste and aroma of baked products. Darker-colored flour may give products a heavier, bitter taste and aroma, while lighter-colored flour may give products a lighter taste and aroma.

Texture: Wheat flour color can affect the texture of bread dough. Darker-colored flour has less elasticity compared to lighter-colored flour.

Appearance of baked products: Wheat flour color can affect the appearance of baked products. Darker colored flour will give products a darker color, while lighter colored flour will give products a lighter color (Yuksel *et al.* 2022).

2.4.2 Protein

The protein content in wheat varies depending on the type of wheat, the region it is grown in, and the growth conditions. Generally, wheat contains a protein content ranging from 10 to 15 percent of the total weight of the grain, and the protein level is affected by the way the wheat is cultivated and various external factors such as temperature, humidity, and soil. Wheat with a high protein content (more than 14 percent) is considered ideal for making baked goods such as bread, pasta, and pizza, and hard red wheat can contain a higher percentage of protein compared to soft wheat. Protein constitutes around 8–15% of the wheat grain, and its quality is mainly affected by the genetic composition of the grain (Stone and Nicolas 1996, Stoner and Savin 1999, De Giorgio *et al.* 2016). Protein in wheat has a significant impact on the quality of flour and baked goods made from it. Protein is the main component responsible for forming gluten, which gives the dough its texture and elasticity and helps retain gases produced during fermentation, resulting in the final structure of the bread. The level of protein in wheat varies according to the variety, geographic region, and other environmental factors. For example, hard wheat usually has a higher protein content compared to soft wheat. High-protein wheat is used in the production of premium bread, pastries, biscuits, and cakes that require a strong structure and good elasticity, while soft wheat is used to produce soft baked goods such as light cakes and pies (Matz 1996). Protein varies in different types of wheat, and it is the best choice to determine the quality of flour due to the centrifugal relationship between the protein component and the quality of bread (De Giorgio *et al.* 2016, Ortolan *et al.* 2017).

Henri-Louis Duhamel du Monceau, a French scientist, was the first to isolate gluten in 1745. Gluten was discovered by displaying a piece of dough on a sheet of paper and washing it with water to remove all other ingredients except for gluten, which the cells that make it up were able to retain. The remaining gluten was then collected and dried.

Alfredo Baccarin is an Italian research scientist who isolated gluten in 1876. Baccarin separated gluten from flour using ethanol (alcohol) and water. He soaked the flour in an ethanol solution for a period of time and then washed it with water, which led to the liberation and isolation of pure gluten. Baccarin's discovery of gluten was a significant breakthrough in understanding the chemical composition of flour and its uses in the food industry. The researcher (Osborne 1907) later divided grain proteins into four groups based on their solubility in different solvents:

Albumins (water-soluble proteins).
Globulins (soluble in a 10% NaCl solution).
Prolamins (soluble in 70–90% ethyl alcohol).
Glutelins (soluble in mildly acidic or basic solutions).

When using the Osborne method to extract wheat proteins, Chen and Bushuk (1970) added another group of proteins related to glutelin's, which they divided into two parts: one part is soluble in 0.05M acetic acid, and the other part is insoluble. Estimating protein content is one of the qualitative measures used to determine the quality and commercial value of wheat. According to Casada and O'Brien (2003), determining the price of grains depends on their protein content. Hard wheat with low protein content is not priced as high as hard wheat with high protein content. As Awad (2000) pointed out, protein content ranges from 10.0% to 14.7% in several varieties of wheat grown in Qatar. She also noted a significant correlation between the quantity of protein and the quantity of gluten in flour, with a correlation coefficient of ($r=0.81$), indicating the importance of estimating flour proteins to estimate gluten content. Sapirstein and Fu (1998) explained that single-chain and multi-chain proteins interact differently with gluten. When flour is mixed with water to form dough, a reaction occurs between the gluten protein in the flour and the water to form gluten, and single-chain proteins play an important role in this reaction. When flour is mixed with water, gluten proteins are released from the flour proteins, and gluten is formed. Single-chain proteins help in the formation and binding of this mass, while multi-chain proteins can negatively affect this process. When yeast is added to the dough, carbon dioxide is released, which enters the gluten and expands to give baked products the desired elasticity and crispy texture.

Single-chain and multi-chain proteins can affect the fermentation process in different ways. Generally speaking, it can be said that single-chain proteins enhance the fermentation process, while multi-chain proteins can inhibit it. The suitability of wheat flour for bread-making is determined by the quantity and quality of its proteins (Wall 1979). Hosen (1969), Orth and Bushuk (1972), and Lookhart *et al.* (1999) have all pointed out that qualitative and quantitative differences between different protein components in wheat kernels of different varieties affect their efficiency in bread making. Payne *et al.* (1985) explained that flour containing low protein content (8–10%) is not suitable for producing high-quality bread. Singh and MacRitchie (2001) noted that water-soluble proteins (albumin) and salt-soluble proteins (globulin) make up 10–22% of the total flour proteins and are of little importance in determining the quality of the flour, but they are important nutritionally due to their high content of essential amino acids (Schofield and Booth 1983). Albumin and globulin are two types of proteins found in flour that affect the quality of bread (Ortolan *et al.* 2017).

Albumin is a water-soluble protein that enhances the elasticity of dough and improves its ability to mix and hold together. It also improves the dough's ability to retain gases released during the baking process, resulting in a soft and cohesive bread.

As for gluten, it is a protein that is not soluble in water and helps to enhance the strength of the dough, giving it elasticity and resistance to stretching and deformation during the shaping and baking process. Gluten is considered one of the factors that helps improve the quality of bread and increase its shelf life.

In general, it can be said that the presence of appropriate amounts of albumin and gluten in flour contributes to improving the quality of bread, making it more flexible, durable, and easier to shape.

2.4.3 Gluten

Gluten is known as a cohesive and elastic protein substance that is responsible for the viscosity of flour. It is also the main component that forms the dough texture of wheat flour after adding water. Additionally, it is an important factor in the unique ability of wheat to produce leavened products (Casiro 2011). Gluten can also be defined as a part of the protein in wheat, barley, rye, or any other varieties that contain gluten. It is insoluble in water and in salt solution at a concentration of mol/L. (Ortolan and Steel 2017). Gluten is a complex protein structure that arises from mixing flour with water during bread-making. The complex composition of gluten is still not fully understood (Al-Sadoun 2016). Gluten accounts for about 78–85% of the total proteins in wheat and is a very large complex consisting mainly of two types of chains: polymeric peptide chains linked by disulfide bonds known as glutenin and individual peptide chains known as gliadins (Caputo *et al.* 2010). Gluten is considered the primary protein responsible for the dough structure of wheat and is also essential for a wide range of technical applications, from strengthening bakery products to developing new protein-based food ingredients (Casiro 2011). Wheat has technological importance related to the ability of dough to stick and expand when flour is mixed with water and other ingredients in the process of making baked goods (Wang *et al.* 2015). Gluten is the main protein in wheat grains and is a good source of plant proteins with good functional properties. However, it is considered a protein with limited nutritional value as it lacks the amino acid lysine in its structure (Elmalinadi 2018). Additionally, the term gluten is also used to refer to the remaining protein residues after starch is isolated from corn. However, corn gluten has a different function compared to wheat gluten. In addition to its role in the specifications of wheat dough, wheat gluten has harmful effects on some individuals, the most important of which is celiac disease, which is caused by alpha-gliadin, a component of gluten. However, the incidence of this disease does not exceed 1% in the general population (Zilic 2013). Gluten protein is characterized by its content of essential amino acids important for human health, which should be provided in meals since it contains all ten essential amino acids (Casiro 2011). However, its content of the amino acid lysine, as well as tryptophan and methionine, is very low (Elmalinadi 2018).

Gluten consists of a complex mixture of proteins that contain numerous multi-peptides that may reach hundreds with different molecular weights (Casiro 2011).

Gluten can be defined as the elastic mass that remains when washing wheat dough to remove starch granules and water-soluble components. As a general rule, hard wheat contains a higher percentage of gluten than bread wheat (Zilic 2013). Gluten is desirable in some wheat products, such as bread, but not in others, such as biscuits. Therefore, choosing the right type of wheat flour for a specific product requires high accuracy and is a challenging task. Bread production requires wheat flour with a high gluten content, while biscuit production requires wheat flour with a low gluten content (Kumar *et al.* 2013). The composition of wheat gluten, its interaction with other wheat components, and its ability to convert wheat flour into an elastic texture are still challenges for researchers. Likewise, learn how to retain gas in the dough and how to convert the dough and expand the bread (Khatkar 2014). Gluten is a mixture of prolamin proteins that are mainly present in wheat, barley, corn, and oats (Balakireva and Zamyatnin 2016), and wheat gluten is the primary indicator of the rheological properties of wheat dough and the texture of wheat baked goods that depend on direct dough processing (Thiele 2003). The properties of elasticity and viscosity (viscoelastic) are attributed to the quality and quantity of gluten proteins, which affect the size, texture, shape, and appearance of bakery products (Singh and Singh 2013).

2.4.4 Humidity

Moisture is considered one of the fundamental parameters for wheat flour, and it affects the physical and chemical properties of the flour and thus its quality. Moisture is measured in percentages (%) and refers to the amount of water present in the flour. The qualitative properties of flour are affected by changes in its moisture content. When wheat flour is dry, it has a high ability to absorb liquids and yields good results in kneading and baking, while if it is too moist, it tends to clump and stick together, making it difficult to knead. In addition, a higher moisture content in wheat flour increases the likelihood of mold and bacterial growth, which can lead to a deterioration in flour quality. Moisture content in wheat flour is controlled during storage and production, and flour is typically stored in a dry and cool place to minimize the effects of moisture. Preservatives such as sorbate and potassium propionate can also be used to extend the shelf life and reduce the likelihood of deterioration due to moisture. (Czaja *et al.* 2020, Zhang *et al.* 2023).

Moisture significantly affects the shelf life of wheat flour. When flour is exposed to moisture, it begins to experience heat buildup and the growth of bacteria and mold, leading to a deterioration in quality, a decrease in nutritional value, and a shortened shelf life. Generally, it is recommended to store flour in a cool, dry, and dark place to minimize exposure to moisture. Flour should also be stored in airtight containers to preserve its freshness and quality for a longer period of time. Pomranz (1988) mentioned that moisture has a clear effect on the shelf life of flour. Flour produced from dry wheat can be stored for a longer period of time if stored correctly, whereas flour produced from moist wheat will be more prone to spoilage during a short storage period.

2.4.5 Permeability

The wheat passage rate through sieves is the measure used to determine the size and distribution of wheat particles. It is calculated by passing a sample of wheat through a set of sieves with different sizes and measuring the percentage of wheat that passes through each sieve. The wheat passage rate through sieves depends on the purpose for which the wheat is used. For example, if the goal is to produce white flour, the wheat passage rate through sieves should be between 70 and 80%, meaning that a large portion of the large particles in the wheat are rejected and separated, resulting in the production of fine flour. However, if the goal is to produce whole wheat flour, the wheat passage rate through sieves should be less than 50%, meaning that all wheat particles, including the outer part of the grains, should be used. The wheat passage rate through sieves varies depending on the type of wheat, its growing conditions, storage conditions, milling method, and other factors. This measure is used in the food industry to determine the different uses of wheat, especially in the production of flour, pasta, and other wheat-based products (Kong *et al.* 2020).

2.4.6 Ash Content in Flour

Ash content in wheat flour refers to its mineral content, which varies depending on several factors such as wheat variety, fertilization, cultivation location, and climate. The majority of minerals are found in the wheat germ and bran, while the endosperm contains fewer minerals. Therefore, if the flour contains a significant amount of bran, it is natural for it to have a higher ash content (Posner and Hibbs 1997). The ash content test is considered one of the most important tests for determining the quality and nature of flour use. It serves as an indicator of the degree of flour extraction, where an increase in extraction rate leads to an increase in ash content and color intensity (Pomeranz and Mattern, 1988). This is due to the significant correlation between the two factors, with a correlation value of (r=0.94) observed for varieties with extraction rates ranging between 66% and 82% (Orth and Mannader 1975). The estimation of ash content is important in determining the efficiency of the separation process between endosperm and bran during milling. In general, high-quality flour has a low ash content, and studies have shown a relationship between the ash content and the color of the flour, where a higher ash content indicates a higher proportion of bran, resulting in a less white color. The ash content ranges from (1.8–2.5%) in whole-grain flour with a 100% extraction rate, while the values are lower in flour with a 72–79% extraction rate (Pomeranz 1971). The acceptable ash content in flour for baking is around (0.5–0.82)% for grade zero flour (Orth and Mannader 1975), while Zainalabedin (1979) set it at (0.35-0.52%) for locally produced bread flour. Studies have also shown a significant correlation between ash content and protein content (Dikeman *et al.* 1982, Zaja *et al.* 2020, Zhang *et al.* 2023).

Ash content in flour determines its extraction rate, as ash content represents the percentage of minerals and salts in the flour after combustion. Therefore, the higher the ash content in the flour, the lower the extraction rate. Estimating the ash content is used to determine the quality of the flour and classify it into different grades. Fine flour is considered to have an ash content of less than 0.55%, while coarse flour is considered to have an ash content ranging from 1.5% to 2%. In general, estimating the ash content provides an idea of the quality and extraction rate of the flour (Czaja *et al.* 2020, Zhang *et al.* 2023). (Matz 1996) noted that ash content is important in estimating the extraction rate of flour because the ash content in bran is estimated to be about 20 times higher than in endosperm, making it a suitable indicator for the efficiency of separating bran and germ from the endosperm.

2.5 Rheological Properties of Flour

The rheology of flour is concerned with the study of the flow and deformation properties of dough and baked goods. These properties include resistance to deformation, elasticity, viscosity, plasticity, shrinkage, and expansion. Rheology is used to understand the behavior of dough and to improve various processes related to baked goods production, including dough mixing, sheeting, fermentation, and baking. Rheology helps bakers develop different products by determining the desired properties of the dough and adjusting the appropriate processes to achieve those properties (Danqiu Ren 2007).

2.6 Effect of Metal Reinforcement on Rheological Properties

Mineral fortification, especially iron fortification, affects the chemical composition of flour. Iron is added to flour in the form of mineral salts, such as iron phosphate, with the aim of increasing the iron content in the diet and improving the properties of flour and baked products made from it. In addition, iron fortification of flour can affect the properties of dough and baked products produced from it, such as dough texture and elasticity, color of baked products, and other nutritional properties. It can also affect the ability of dough to rise, withstand, and retain moisture (Akhtar *et al.* 2009).

2.7 Effect of Phytic Acid on Iron Availability

Phytic acid is a monocarboxylic acid commonly used as a leavening agent in the baking industry. It works to improve dough properties and enhance the quality of the final product. Fumaric acid reacts with the proteins in flour to form a network of cross-linked proteins that help to improve the dough's elasticity and increase the final product's volume. Additionally, fumaric acid also improves the dough's cohesiveness, extensibility, and elongation properties, resulting in improved texture and flavor characteristics. Fumaric acid is also used to improve the shelf life of the final product, as it helps to stabilize colors and flavors and reduces changes in the chemical composition of the baked goods during storage (Toree *et al.* 1991).

Phytic acid is concentrated in the outer bran layer of wheat grains and surrounds the inner portion of the grain. Phytic acid is considered an antinutrient as it can bind to certain minerals like iron and zinc, preventing their absorption by the body. Therefore, the processing of grains is carried out to reduce the level of phytic acid before using them in food and food production (Lopez *et al.* 2003).

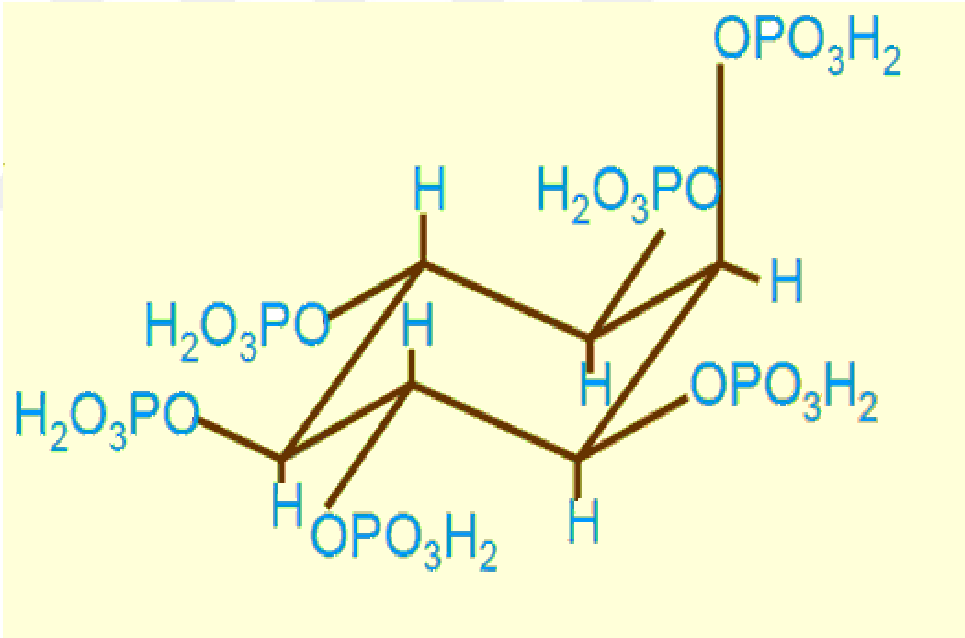

Figure 2.1 Structural formula of phytic acid

2.8 Effect of Manufacturing Processes on Phytic Acid

The manufacturing processes of flour can affect phytic acid, as the outer layer of wheat grains is removed during milling, leading to a decrease in phytic acid concentration in the resulting flour. Additionally, washing, cleaning, drying, and storing the grains can also affect the concentration of phytic acid in the grains, which in turn affects the concentration in the resulting flour. Generally, measures that reduce damage to the grains during harvesting, storage, and manufacturing can improve the concentration of phytic acid in flour (Lotfi *et al.* 1996).

The fermentation process affects phytic acid in flour in various ways. During fermentation, yeast is added to the dough, starting the fermentation process where sugars are converted to lactic acid and carbon dioxide. Yeast uses lactic acid to obtain the energy needed for growth and reproduction, thereby reducing the acidity level in the dough, leading to a reduction in the percentage of phytic acid in the flour.

It is worth noting that other manufacturing processes, such as wet milling and iron fortification, can also affect phytic acid in flour. These processes can increase the amount of phytic acid in flour and affect its stability during storage (Spiller 1993).

2.9 Fortification of Wheat Flour with Iron

Iron fortification of wheat flour is the addition of iron to wheat flour as a nutritional supplement. This is done to improve the iron content in the diet, as iron is an essential nutrient that plays a crucial role in metabolism and the growth of the body and helps prevent iron deficiency and related diseases such as anemia. Wheat flour is typically fortified with iron by adding iron compounds to the flour during the manufacturing process. (WHO/EMR 1998).

Fortification of wheat flour with iron began in the 1960s, when governments and global health organizations realized that iron deficiency could lead to serious health problems such as anemia. Since then, fortification of wheat flour with iron has become very common worldwide (Task Force for Child Survival and Development, Atlanta 1992, Ranum 2000).

Table 2.1 Fortification of wheat flour with iron for different countries in the world (Maberly 2002)

Country	Wheat Flour Cons. Ton/yr	Per Capita Cons. Kg/yr	Fortification	Iron Fortificant	Level (in standard) mg/kg
Argentina	3226000	87.0	Yes-Aug. 2002	Ferrous Sulfate	30 as Fe
Belize	18200	53.2	Yes	Reduced Iron	60
Bolivia	390000	48.7	Yes	Reduced Iron	60
Brasil	7656000	42.0	Voluntary	Reduced Iron	42
Caribe(Barbados, Curacao, Grenada, Guadalupe,Guyana, Haiti, St. Vincenty Grenadines)			Yes (Caricom Agreement)	Not defined	Not<29 or >46
Chile	1312069	106.3	Yes	Ferrous Sulfate	30
Colombia	1090592	26.5	Yes	Reduced Iron	44
Costa Rica	194000	46.1	Yes	Reduced Iron	60
Cuba			No (Info N/A)		
Ecuador	469630	35.8	Yes	Reduced Iron	55
El Salvador	220000	33.7	Yes	Reduced Iron	55
Guatemala	324600	27.1	Yes	*Reduced Iron	55
Honduras	244120	35.7	Yes	*Reduced Iron	55
Jamaica	149317	58.0	Yes-Voluntary	Reduced Iron	44
Mexico	4152776	38.8	Voluntary	** Reduced Iron	35
Nicaragua	83630	15.7	Yes	Reduced Iron	55
Panama	103600	35.2	Yes	Reduced Iron	60
Paraguay	171645	29.7	Yes	Ferrous Sulfate	45 as Fe
Peru	1002002	38.0	Yes	Reduced Iron	28
Puerto Rico			Yes	Same as USA	
Republica Domincana	172014	19.7	Yes Voluntary	As per Codex	
Surinam	27705	61.0	Yes	Caricom	
Trinidad Tobago	N/A		Yes	Caricom	
Uruguay	243300	72.3	No	N/A	
Venezuela	873000	39.0	Yes	Ferrous Fumarate	20 as Fe

*Ferrous Fumarate is also allowed in the legislation, **Iron source not defined

2.10 HPLC technology to identify dyes and additives

HPLC technology is a chromatography method that relies on two phases: the stationary phase and the mobile phase. There are several types of HPLC separation methods based on the principle of separation. If the separation is based on molecular weight, it is called (size exclusion chromatography." If the separation is based on hydrophobicity, it is called reverse-phase chromatography. If the separation is based on the distribution of compounds between the stationary and mobile phases, it is called partition chromatography. If the separation is based on the predominant charge in the separated compounds, it is called ion exchange chromatography. Finally, if the separation is based on the affinity of the compounds to the surface of the support material in the column, it is called adsorption chromatography (Regnier and Gooding 1980).

HPLC chromatography is a successful and widely used technique for distinguishing and diagnosing grain varieties. Since Bietz (1983) proved that stored proteins could be analyzed using HPLC and used to tell the difference between grain varieties, many more studies have shown that this method can tell the difference between wheat varieties (Bietz and Cobb 1985, Buranouf *et al.* 1983), barley varieties (Allison and Bain 1986, Wingad *et al.* 1986, Marchylo and Kruger 1984), and yellow maize varieties (Paulis and Bietz 1986).

HPLC chromatography has several advantages over other techniques used to distinguish varieties, such as polyacrylamide gel electrophoresis (PAGE) or SDS (SDS-PAGE). It is a fast and sensitive technique, and it is easy to estimate the amounts of protein components from the separation diagram (quantitated). The HPLC method can also be programmed and automated. The matching and distinction of separation diagrams is based on the retention time, the number, and the area of peaks for each variety. Computer programs can be used to match information between unknown varieties and a known standard variety (Wieser *et al.* 1998, Scunlon *et al.* 1987).

SE size exclusion and RP reverse phase chromatography are accurate and quick ways to separate wheat proteins (gliadins and glutenins) so that different types can be identified, the molecular weight distribution of wheat proteins can be studied, and quality traits of wheat and flour can be predicted based on the proteins. Other types of chromatography have also been used in this field, where researchers (Huebner and Wall, 1966) separated wheat proteins using ion exchange chromatography and found differences and non-similarity in gliadins among different wheat varieties. The same researchers (Huebner and Wall 1976) used the gel filtration method for discriminating and studying wheat varieties by analyzing the peaks of separated protein components. Locally, researchers such as Sahi (1981) were able to separate soluble and insoluble wheat proteins using the gel filtration method. Bietz (1983) pointed out the effectiveness of the reverse-phase RP-HPLC technique in differentiating and dividing wheat varieties based on certain criteria, and this technique also helps in distinguishing between strains in breeding and improvement programs. Lookhart *et al.* (1986) confirmed the role of RP-HPLC in distinguishing between varieties and environmental forms, while Ng *et al.* (1989) used HPLC and electrophoresis on polyacrylamide gel with or without SDS to analyze proteins from 68 Canadian wheat varieties for classification purposes. The researcher explained that there are some soft wheat varieties that are similar in appearance and also in some qualitative or chemical characteristics, making it difficult to distinguish between them using some electrophoresis methods such as PAGE and SDS-PAGE. However, they can be distinguished using the HPLC technique based on the number, areas, and heights of some peaks that take the same amount of time to appear from the column. The researcher emphasized that the HPLC technique can be useful in distinguishing wheat varieties, registering and adopting new varieties, and verifying seed purity in terms of genetic traits, as well as in qualitative and genetic studies of the wheat crop.

In 1999, Hubner and his team used HPLC to separate the protein parts of eight types of soft red and white wheat. They then looked at the statistical relationship between these protein parts and some chemical, physical, and quality factors, like the biscuit diameter, protein content, and sedimentation value. They discovered that environmental factors and wheat variety also have an impact on the amount of protein components. By studying the separation patterns of gluten proteins and their quantities using HPLC, the mixing ratio of different varieties can be determined. (Marchylo *et al.* 1988) demonstrated a significant correlation between the area of separated peaks and the concentration of varieties present in a certain blend of wheat grains. This is crucial for the operation of silos and milling facilities. HPLC technology can be used to detect heat-exposed grain proteins. (Windemann *et al.* 1986) were able to diagnose wheat gluten in gluten-free foods for celiac disease patients with greater accuracy and efficiency than using ELISA, which is not suitable for heated foods. This technology can also identify the presence of wheat and rye flour in such foods at levels up to 2%, and even in a food mixture containing over 50% milk, wheat flour can be detected at levels up to 5%. The efficiency of wheat protein separation by HPLC technology is equal to or better than other separation techniques, as evidenced by the number of peaks that can be separated. Bietz *et al.* (1983) were able to separate gliadin into more peaks than one-dimensional electrophoresis.

The study of storage proteins in wheat and their relationship with different quality standards can be conducted using traditional methods. However, such methods require a long time and large sample sizes compared to the HPLC technique, which can analyze proteins more quickly and from smaller samples. This technique is a synonym for using the Single-Kernel Characteristics System (SKCS) (Hubner and Bietz 1993, Hubner and Bietz 1985). This system is currently used in advanced countries to classify and grade new varieties of wheat by studying the physical characteristics of raw wheat, such as hardness, moisture, grain diameter, and grain weight, based on a small number of grains (300 grains per specific variety). Then, a statistical study is conducted between these characteristics and the quality of the resulting flour and bread (Ohm *et al.* 1998).

2.11 Additions to Flour

Preservatives are used in the production of wheat and other foods to prevent spoilage and increase their shelf life. Although these preservatives are generally considered safe, the bleaching agent is used in wheat production to improve its color and make it more attractive to the eye. Some studies indicate that it may have some harmful effects on human health if consumed in large quantities. The bleaching agent used in wheat production contains azodicarbonamide, a substance that has effects on the human digestive system. Studies indicate that consuming excessive amounts of this substance can cause some negative effects on health, such as increasing the risk of allergies and digestive system-related diseases.

2.12 Bread Improvers

There is currently a lot of controversy surrounding bread improvers, as these food additives can pose a risk to consumer health. Therefore, it is advisable to avoid consuming bread that has been enhanced with bread improvers. Bread improvers are substances added to dough ingredients to improve bread quality, prolong shelf life, enhance nutritional value, and enhance the sensory properties of bread. These additives can come in either powdered or gel form.

Bread improvers are classified according to their functional roles, as there are bleaching agents, oxidizing agents, emulsifiers, vitamins, and antifungal agents. These additives have many functions, including improving the quality of the flour, enhancing the mechanical properties of the dough, increasing water absorption, improving fermentation properties, giving a consistent shape to the bread, preserving the freshness and giving it a sponge-like texture, giving the crumb a white color, and giving the crust a golden color.

Azodicarbonamide (ADA) was previously used as a blowing agent in rubber products, and foamed polyethylene was allowed in other food packaging applications (Gregory *et al.* 2008). In some countries, ADA has also been approved as a food additive for use as a flour bleaching agent and dough conditioner, but in October 2003, two reports from the European Food Safety Authority indicated that foamed polyvinyl chloride (PVC) liners, manufactured using ADA, were found to be a source of contamination in a variety of sliced foods. It is commonly used as a flour additive at a maximum of 45 mg/kg in flour in the United States, Canada, and Asia (Cereal *et al.* 2004). ADA is considered banned in Australia and Europe as a food additive. In Singapore, the use of ADA can result in up to 15 years in prison and a fine of up to $450,000. As a flour bleaching agent and improver, ADA may cause allergies in people who are allergic to other azo compounds. The consumption of ADA also leads to an increased allergic reaction to other food ingredients. ADA is stable in dry flour, but it reacts with moist flour as an oxidizing agent, and the main reaction product is biurea (not urea), which is unstable during heating (Becalski *et al.* 2006, Stadler *et al.* 2004). SEM shows limited genotoxicity in the laboratory and causes cancer and tumors (Becalski *et al.* 2004).

Azodicarbonamide (ADA) is an orange-yellow crystalline substance used as a foaming agent in plastics and as a food additive (a bleaching agent for wheat flour and dough improver). According to the joint meeting of the Food and Agriculture Organization and the World Health Organization on Food Additives (JECFA), the maximum treatment concentration for wheat flour is 45 mg/kg; however, its use is not permitted because it is unspecified. Therefore, imported products may contain unspecified ADA, and analyzing ADA in flour and flour products is necessary for monitoring imported foods (Yasui *et al.* 2016).

Antioxidants are usually added to flour to accelerate its natural maturation process. It is known that their action in improving dough is due to the oxidation of the sulfhydryl groups present in the gluten proteins in flour. These agents may participate in bleaching only, in both bleaching and dough improvement, or in dough improvement only. Flour bleaching primarily involves the disruption of the double-conjugated carotenoid system to a less-conjugated colorless system. Benzoyl peroxide is one of the most commonly used oxidizing agents in wheat flour, and its bleaching or color removal process appears to have no effect on bread properties. Despite its use in many countries, there are not always regulations regarding acceptable amounts and conditions of use. Benzoyl peroxide is a free radical initiator, and carotenoid oxidation is produced by a typical free radical mechanism. The reaction products between certain peroxyl radicals and carotenoids have been studied in a variety of chemical systems, but not all reaction products have been identified (Saiz 2001).

Different types of oxidizers are used in bread-making, such as azodicarbonamide, calcium peroxide, potassium bromate, potassium and calcium iodate, and ascorbic acid. These oxidizers are used in bread dough systems to make flour and bread crumbs whiter and to help gluten proteins form a disulfide bond, which makes the dough stronger by changing the gluten proteins. Oxidizing agents improve the gas retention properties of the dough, which generally leads to an increase in the size of the final loaf. Potassium bromate is often used in commercial bread-making because it acts as an oxidizing agent in dough systems at high oven temperatures and also works as a bleaching agent in flour.

Potassium bromate ($KBrO_3$) is a white, crystalline, toxic solid that exists in the form of granules and crystals. It has a molecular weight of 167 g/mol and a melting point of 350 degrees Celsius. It can decompose at 370 degrees Celsius, releasing oxygen, and its density is 3.27 g/cm3. This oxidizing substance must be handled with great care. It is soluble in water but insoluble in alcohol. Swallowing this substance can cause vomiting, blood disorders, and internal injuries.

Potassium bromate ($KBrO_3$) has been widely used as a flour treatment agent due to its bleaching effect. It is also used as a food additive to improve the taste of meats, vegetables, and other foods. However, $KBrO_3$ has been proven to be toxic to humans, harming kidney and brain function as well as affecting hearing, tissues, and other organs. Some studies have also suggested that $KBrO_3$ may have carcinogenic and mutagenic effects. The Joint FAO/WHO Expert Committee on Food Additives has recommended against using $KBrO_3$ as a food treatment agent and has advised a temporary guidance value of 10 nanograms per milliliter of $KBrO_3$ in drinking water (Ahmad *et al.* 2012, Farmany *et al.* 2014).

Potassium bromate ($KBrO_3$) is a slow-acting oxidizing agent that improves gluten strength when the dough is at its weakest point. During the baking process, potassium bromate breaks down into a stable compound of potassium bromide (KBr). Sometimes this reduction is incomplete, resulting in residual potassium bromate in the bread. Residual potassium bromate is a source of oxidative damage and is not considered beneficial for health. Research has shown that potassium bromate is a carcinogenic substance, and therefore the bread-making industry is looking for an alternative to potassium bromate. The US Food and Drug Administration has classified ozone as GRAS (generally recognized as safe). Subsequently, many countries banned or reduced the use of $KBrO_3$ in food production. Therefore, it is necessary to develop a simple, rapid, and sensitive method for detecting KBrO3 to improve food quality and protect consumers (Kaya and Topaktas 2007).

3. MATERIALS AND METHOD

3.1 The Devices and Chemicals Used in Research

3.1.1 The devices used in the research

Table 3.1 Laboratory devices and materials used, their manufacturers, and country of origin

T	Device name	Manufacturing company	Country of origin
1	Sensitive balance	Shimadzu	Japan
2	Water Distillator	Gallenkamp	England
3	Kjledahl	Memmarnt	Germany
4	Muffle Furnace	Nabertherm GmbH	Germany
5	Electric oven	Humboldt	USA
6	HPLC	Sykam	Germany
7	Fridge	Vestel	Turkey
8	Disposable Syringes	Misawa	Japan
9	Laboratory mill	-	-
10	Small electric mixer	-	-
11	Various laboratory glassware	-	-
12	Center Fusion	Perten	Sweden
13	Glutomatic	Perten	Sweden
14	Electric sieve device	Beko	Turkey
15	Electric mixer	Vestel	Turkey
16	Laboratory mill	Beko	Turkey
17	Hand blender	Qulgg	China
18	Various glassware	-	China

3.1.2 Chemicals

Necessary chemicals were used to conduct the research, as shown in the Table 3.2.

Table 3.2 Chemical substances, their manufacturers, and country of origin

T	Subject Name	Manufacturing company	Country of origin
1	Sulfuric acid	BDH	England
2	Sodium hydroxide	BDH	England
3	Ethyl alcohol	Biomeriux	France
4	Hydrochloric acid	Biomaghrab	England
5	Petroleum ether	BDH	England
6	Boric acid	BDH	England
7	Perchloric acid	BDH	England
8	p - hydroxy benzoic acid	HBA	
9	Methyl ester	HBA	
10	Ethyl ester	HBA	
11	Propyl ester	HBA	

3.2 Collecting and preserving specimens:

12 specimens of wheat varieties were collected, of which 11 were imported from different origins and available in the local markets in the city of Ramadi, Anbar Province, Iraq, on March 27, 2022, in addition to the approved local variety (control sample).

After collecting the samples, they were placed in 5 kg plastic containers and stored in a dry, dark place at room temperature until laboratory tests and sensory evaluation were carried out.

Table 3.3 Abbreviations for Study Parameters, Their Trade Names, and Sources.

Treatment	Variety	Origin
F1	Fakher	Kuwait
F2	Al khir	Turkey
F3	Zer	Turkey
F4	Al raid	Jordan
F5	Rasha	Jordan
F6	Al konooz	Turkey
F7	Barakat Alferdos	Iraq
F8	Mazariana	Iraq
F9	Banetti	Turkey
F10	Wisam	Turkey
F11	Babil	Turkey
F12	Local	Iraq

Chemical tests

- Humidity
- Protein
- Ash
- Color
- Wet glutin
- Dry Glycein
- Additives
- Bleached materials
- Reinforced materials

3.3 Moisture Determination

Humidity was estimated as stated in AACC 2010 No. (19.01–44) using the oven at a temperature of 135 °C for sprouted and non-germinated wheat flour until the weight is stable.

3.4 Ash Determination

The percentage of total ash in grain samples was estimated in the central laboratory of the College of Agriculture, Anbar University, as the weight (1g) of each sample was placed in a ceramic box and then placed in a device (Muffle Fumace) at a temperature of 600 degrees Celsius for a period of five hours, after which the sample was weighed after burning and the percentage of ash was calculated. Then the ash percentage of wheat flour was estimated as stated in AACC (2010), numbered (03.01–08), sample weight 1 gram, using the incineration oven at 550 °C.

$$\text{ash ratio\%} = \frac{\text{Weight of the container after burning} - \text{Weight of the container Empty}}{\text{sample weight}} \times 100$$

3.5 Fat Determination

The fat percentage was estimated for flour as stated in AACC (2010) No. 10.01–30 by using the Soxhlet method and using hexane as a solvent.

$$= \frac{\text{Weight of flask with fat after extraction} - \text{Emptyflask weight before extraction}}{\text{sample weight}} \times 100$$

3.6 Fiber Determination

The fiber content was estimated for wheat flour as stated in AACC (2010) No. 10.01-32. 2 g of the defatted sample was digested with 1.25% sulfuric acid and 1.25% sodium hydroxide..

$$\text{fiber percentage} = \frac{\text{The weight of the lid with the sediment after drying} - \text{the weight of container with ash after burning}}{\text{sample weight}} \times 100$$

3.7 Protein Determination

Estimation of the protein percentage of wheat flour as stated in AACC (2010) No. 10.01–46 by digesting the sample with concentrated sulfuric acid and calculating the quantity of nitrogen.

$$\text{Nitrogenpercentage} = \frac{\text{volumeHCL for molarity (ml) x molarity 0.05 x } 0.014 \times 100}{\text{sample weight (gm)}}$$

Total protein percentage = Nitrogen output ×6.25

3.8 Carbohydrate Determination

The percentage of carbohydrates was estimated by calculating the difference after obtaining the percentages of protein, fat, fiber, and moisture and subtracting them from 100%. The difference represents the percentage of carbohydrates.

3.9 Qualitative Traits

3.9.1 Wet gluten %

The wet gluten of the grains was estimated in the Quality Control Department at the General Company for Grain Processing, which belongs to the Ministry of Commerce, according to the following steps based on its instructions:

a. The flour sample of the grain to be tested was mixed well.

b. 10g of the sample was weighed.

c. The sample was placed in the plastic washing container using the soft inlet (88 microns) and placed in a 2% salt solution (obtained by dissolving 20g of table salt in 1 liter of distilled water).

d. The first stage of washing was carried out automatically in the Wash Chamber Glutomatic device.

e. The remaining part of the sample after washing was transferred to the plastic washing container using the coarse sieve.

f. After the second stage of washing in the automatic device, the remaining part of the sample was transferred to the centrifuge device for the purpose of separating weak gluten from strong gluten.

g. The portion of the sample passing through the sieve of the centrifuge device was weighed, and the results representing the wet gluten percentage were recorded.

3.9.2 Coefficient of gluten (%)

Continuing with the steps for measuring wet gluten, the weight of the remaining part is added to the weight of the passing part, and the gluten index is calculated using the following equation: Gluten index = (total weight - passing weight) / total weight x 100

Samples were tested, and the gluten index was measured in the Quality Control Department laboratories of the General Company for Grain Processing, affiliated with the Ministry of Trade in Baghdad.

3.9.3 Dry gluten (%)

The total sample was placed in a moisture meter dryer, and then the sample was weighed after the loss of moisture in the drying process, which represents its dry gluten content. The dry gluten content of the grain samples was estimated in the Quality Control Department laboratories of the General Company for Grain Processing, which is affiliated with the Ministry of Trade in Baghdad.

3.10 Estimates of Additives and Preservatives in Flour

The preservatives and additives of various types of flour were determined using a Shimadziu high-performance liquid chromatography (HPLC) device of Japanese origin in the laboratories of the Ministry of Science and Technology in Baghdad, according to the method proposed by Iberl and Knobloch (1990). The sample, weighing 25 grams, was dissolved in a mixture of 50 ml of 70% ethanol and 30% distilled water, then placed on a shaker for an hour and subsequently centrifuged at high speed for 10 minutes. Only the supernatant was taken and filtered again (using a 0.45 syringe filter) before being injected into the HPLC device according to the analytical program (column/C18, mobile phase/CAN:H2o -80:20, flow rate/1 ml/min, injection/100 ul, detection/235 nm).

3.11 Baking Test

Dough Method Straight, using the standard method (10–10) of AACC (1998) with some modifications, was used to prepare loaf bread. The mixing ingredients used to prepare the bread were 300 g flour, 6 g sugar, 4.5 g salt, 3 g dry yeast, and water as needed. The yeast was activated first with half the amount of sugar (3 g) and 20 ml of water at a temperature of 30 °C for 10 minutes. The flour and other mixing ingredients were then placed in a mixer and left to mix for two minutes. The activated yeast was added first, followed by the remaining quantity and the necessary water, until the dough reached full maturity. The dough was then transferred to the fermentation chamber at a temperature of 32 °C and a humidity of 75–85% for 35 minutes for primary fermentation. The dough was then cut into pieces weighing 150 g each, placed in standard molds, and returned to the fermentation chamber at the mentioned temperature and humidity for 60 minutes for secondary fermentation. The bread was baked in an electric oven at a temperature of 250 °C for 20 minutes with a source of steam. After the baking process, the bread loaves were cooled, weighed, and their volume measured using the seed displacement method. The baking process was repeated three times.

3.12 Sensory Evaluation

Seven evaluators graded samples using a sensory evaluation form that the General Company for Grain Trade Quality Control Department created in accordance with the regulatory guide for laboratory work (1984). Fifteen sections evaluated the external and internal sensory characteristics of the product loaf, including experts from the Food Science department faculty and some fourth-year students, based on the sensory evaluation form in the Food Science department/College of Agriculture/University of Anbar. Loaf samples were produced using flour and kept at room temperature for two hours before the sensory evaluation process. The suggested global sensory limits are: excellent (90% or above), good (80–90%), acceptable (70–80%), and unacceptable (below 70%) (Camargo 1987).

Table 3.4 Sensory Evaluation Form Used in the Study (Zubaidi 2009)

Adjective	Class	The Transaction 1	The transaction 2	The transaction 3	The transaction 4	The transaction 5
Specific size	30					
Shell color	10					
Shape symmetry	5					
Consistency of the baking process	5					
Pulp granularity	10					
Pulp color	10					
Pulp tissue	10					
Taste and flavour	20					
The total	100					

3.13 Statistical Analysis

The data was analyzed using a completely randomized design (C.R.D.) in a one-way direction, and significant differences between means were tested using the Duncan's test at a significance level of 0.05. The data analysis was performed using the statistical software SPSS, as described in Steel and Torrie (1960).

4. RESULTS AND DISCUSSION

4.1 Pre-production Accuracy Inspection

4.1.1 Flour color

The color of the produced flour may vary depending on the type of wheat and its processing method. Factors such as soil, weather, and agricultural practices can also affect the color of the flour. Annex (1) shows the results of the variance analysis for the studied traits. If there were significant differences ($P<0.05$) between the treatments under study, Figure 4-1 shows that treatment F11 (Babil) recorded the lowest level of the trait at 85.37, while treatment F8 (Mazariana) recorded the highest average for the flour color trait at 91.40. It did not differ significantly from the other treatments due to the presence of additives such as bleaching and incomplete substances, which give the bright white color in most treatments. However, these substances were not available in treatment F11, which recorded the lowest level of the trait, and the reason for this may be attributed to the genetic composition of the wheat used in the treatments. This is consistent with what was mentioned by Zhai *et al.* (2018). The absence of a bright white color in treatment F11 may be attributed to the fact that the ground flour is made up of several types of grains in addition to wheat.

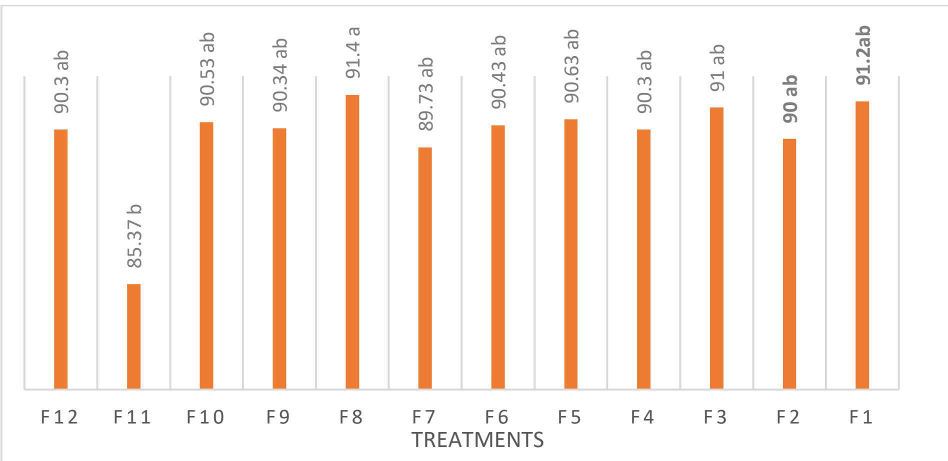

Figure 4.1 The values of the recorded white color in the treatments

4.1.2 Protein

Protein is one of the important components in wheat flour that is related to determining the nature of its final use. Wheat proteins were among the first proteins studied since 1745. Annex (1) shows the results of the analysis of variance for the studied transactions, where significant differences with $P<0.05$ were observed between the studied transactions. Figure 4-2 shows that transaction F7 (Barakat Alferdos) recorded the highest mean for total protein content at 14.067, surpassing all other transactions. Transactions (Alkhir) F2, (Zer) F3, and (Babil) F11 followed with (13.300, 13.033, and 13.367), respectively. Transactions (Fakher) F1 and (Rasha) F5 recorded the lowest mean for total protein content at 11.233 and 11.300, respectively. The reason for this significant difference may be due to the variation in the quality of the transactions used in making the dough or the effect of additives that enhance protein quality. The results agreed with Dencic *et al.* (2011), who confirmed that the protein percentage is directly affected by the quality and classification of the wheat.

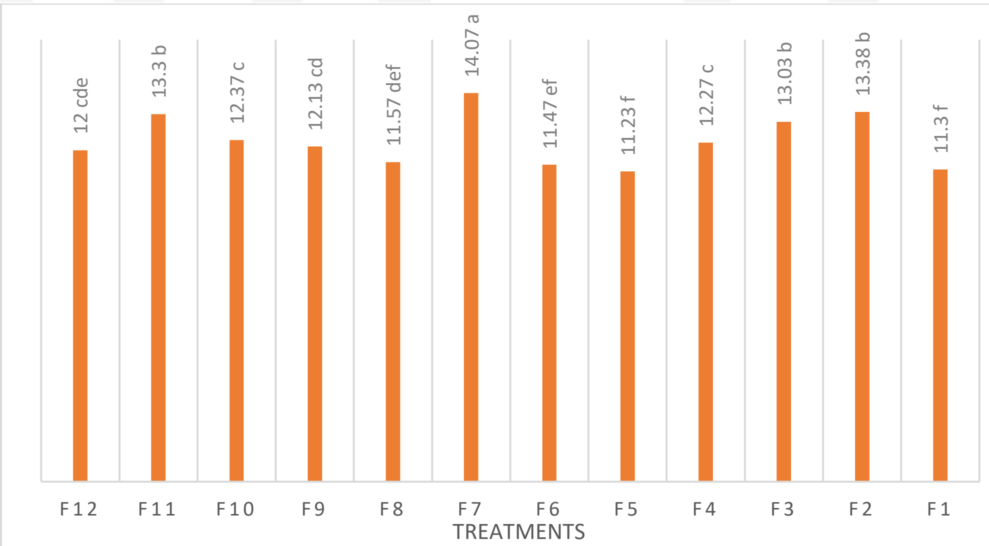

Figure 4.2 The values of the recorded protein in the treatments

4.1.3 Humidity

The moisture content of wheat refers to the percentage of water present in the grain. Moisture content is used to evaluate the quality of grains and to store and transport them. When the moisture content is too high, it makes the wheat more susceptible to mold and spoilage. Conversely, if the moisture content is too low, it affects the milling process and increases the percentage of mustard and impurities in the wheat. Annex (1) shows the results of the variance analysis of the studied variables, revealing significant differences at $P<0.05$ among the treatments under study. Figure 4-3 shows that treatments F1, F7, and F10 had the highest moisture content averages, reaching 12.833, 12.967, and 13.067, respectively, surpassing all other treatments. Treatment F6 recorded the lowest average compared to other treatments, reaching 10.967. The reason for these differences may be due to the storage, transportation, and wheat milling processes. The flour that was tested did not know the methods of its manufacturing, transportation, or storage. These results are consistent with those of Zaja *et al.* (2020) and Zhang *et al.* (2023), who confirmed the need to identify manufacturing, transportation, and storage methods.

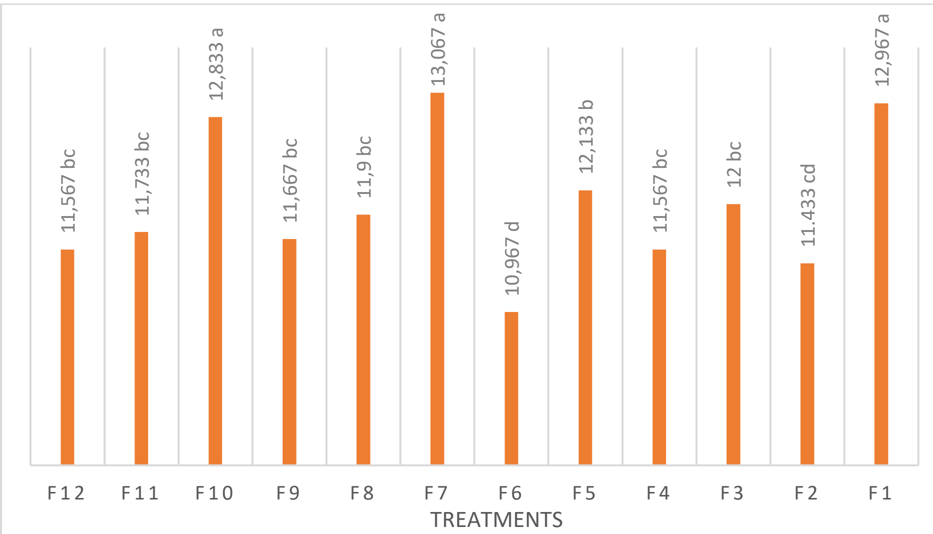

Figure 4. 3 The values of the recorded humidity in the treatments

4.1.4 Permeability

Wheat permeability through the sieve is the measure used to determine the particle size and distribution of wheat. It is calculated by passing a sample of wheat through a series of sieves of different sizes and measuring the percentage of wheat that passes through each sieve. Wheat permeability through the sieve varies depending on the type of wheat, its cultivation conditions, storage status, milling methods, and other factors. Annex 1 shows the results of the variance analysis of the studied treatments, indicating significant differences at P <0.05 among the treatments under study. Figure (4- 4) shows that treatment F5 recorded the highest permeability level (99.833), outperforming all other treatments, while treatment F11 recorded the lowest level of this trait (66.4667). The reason for this is that grinding whole wheat grains with other varieties of grains in the same class prevents the bran from flowing through the sieve, resulting in lower permeability. The results agreed with Kong *et al.* (2020).

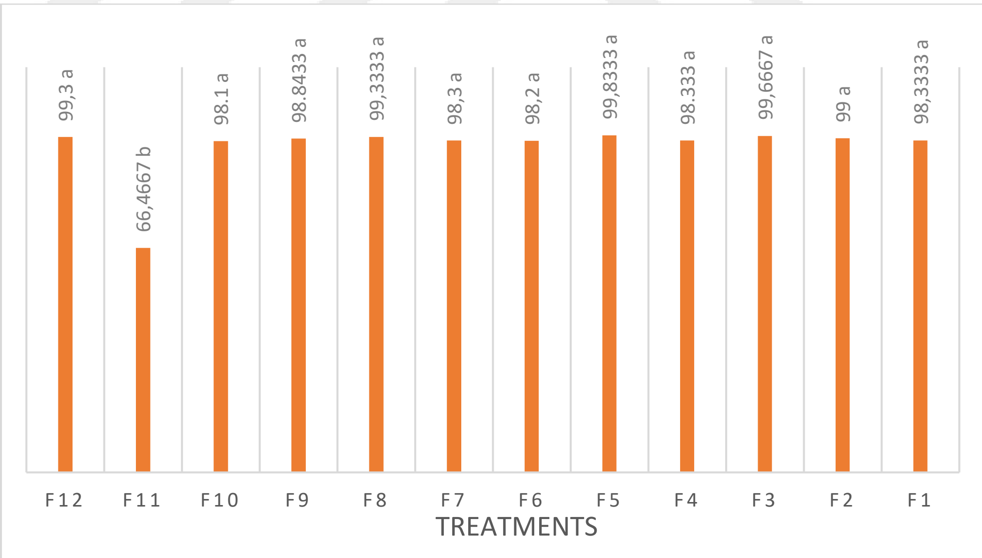

Figure 4.4 The values of the recorded permeability in the treatments

4.1.5 Ash

The ash content in wheat products reflects the amount of mineral substances present in the grains. The ash content is considered an indicator of the nutritional quality of flour, as it represents the mineral content in the produced flour. Annex 1 shows the results of the analysis of variance between the studied treatments, where there are significant differences (P<0.05) between the treatments under study. Figure 4.5 shows that treatment F11 recorded the highest level of ash content, reaching 1.80, while the rest of the treatments recorded different and varied percentages. Treatment F8 recorded the lowest level, reaching (0.588) for ash content. The reason for this variation is due to the genetic composition of wheat and the added materials that improve the quality of the treatment and the milling process. The results are consistent with what was mentioned by (Czaja *et al.* (2020), Zhang *et al.* (2023), and the California Wheat Commission (2001) who confirmed that the ash content is affected by the milling process and supporting materials. The reason for the superiority of treatment F11 may be due to the fact that this flour was produced by grinding the whole grain of wheat, which is not separated from the bran, where most of the minerals in wheat are concentrated (Ngozi 2014).

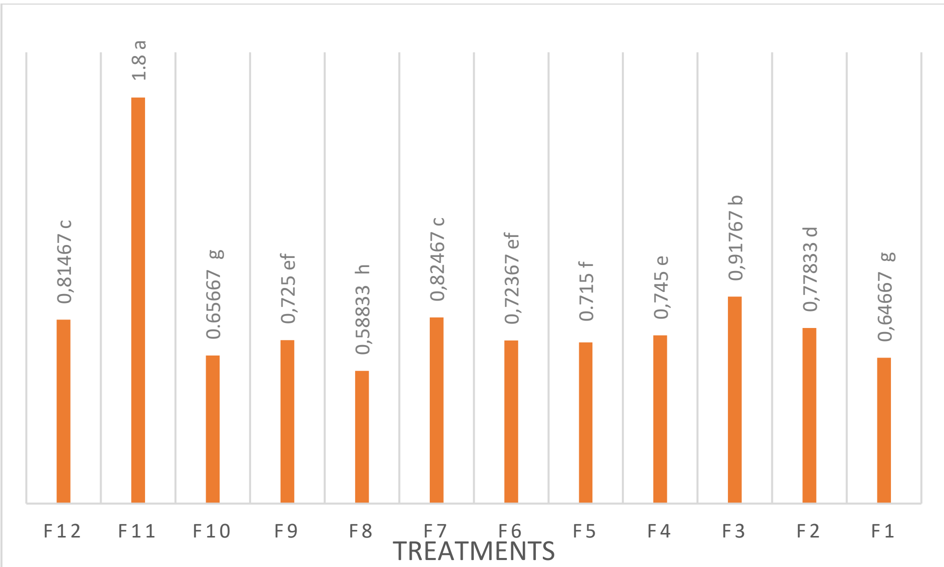

Figure 4. 5 The values of the recorded ash in the treatment

4.2 Sensory evaluation of manufactured white bread

Food product quality is usually associated with sensory evaluation. For this purpose, experienced evaluators in this field are used, and the evaluation result usually reflects consumer preferences for the food, product acceptability, and desire to consume it. Many basic specifications have been developed for evaluating any food product, and flour is evaluated based on the type and quality of the bread produced. Based on this, the value of flour in this study was assessed through:

4.2.1 Shell color

Annex (1) shows the results of the analysis of variance for the studied treatments, which revealed high significant differences ($P<0.05$) among the treatments. Figures (4-6) show that treatment F6 had the lowest level of the trait, which was 7.70, while treatment F7 recorded the highest average in the crust color trait, reaching 9.80. The reason for this can be attributed to the type of flour used, the preparation method, and the oven temperature. These results are consistent with what was mentioned by Rybalka *et al.* (2020). The color of the crust depends on the type of flour used, the dough preparation method, and the oven temperature used during the baking process. If the outer crust of the bread is light in color, it indicates that the bread is not sufficiently matured, while if it is dark in color, it indicates that the bread has matured sufficiently.

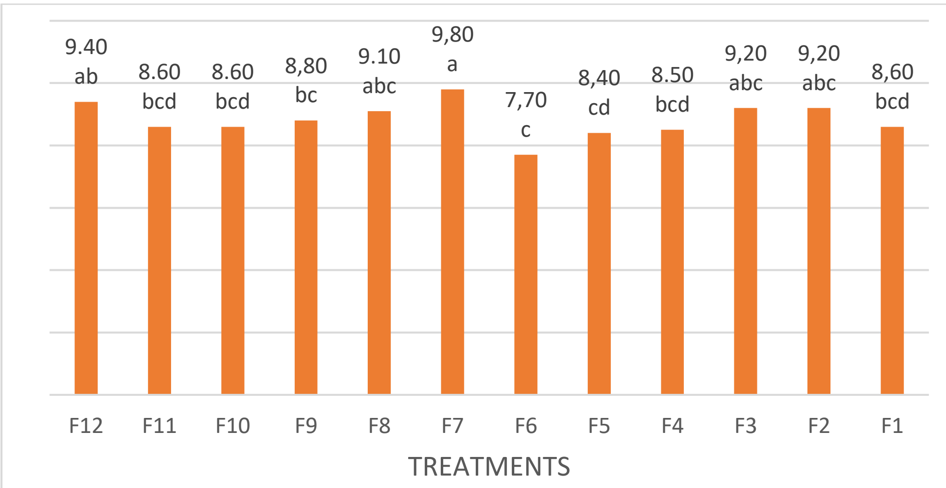

Figure 4.6 The values of the recorded shell color in the treatments

4.2.2 Shape symmetry

Appendix (1) shows the results of the analysis of variance for the studied treatments. The results indicate no significant differences between the treatments. Figure 4-7 displays the values of the treatments, which are close and consistent. This may be attributed to the even distribution of heat during the baking process, the use of equal amounts of dough, and the proper distribution of dough in the bread mold. This is consistent with what was mentioned by Ammar *et al.* (2000) regarding the consistency of bread shape. The consistency of shape in bread after the manufacturing process means that the bread has acquired a consistent and homogeneous shape in all its parts.

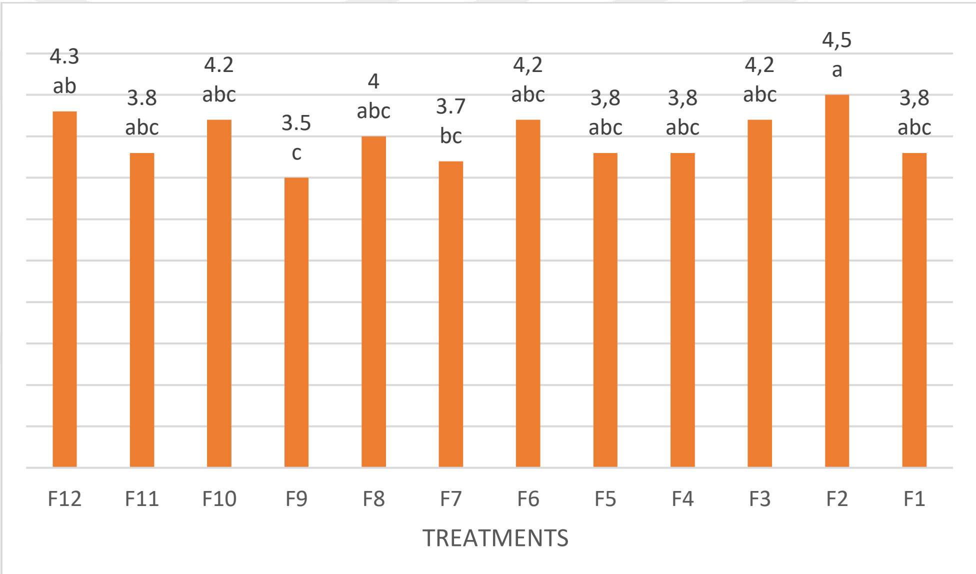

Figure 4.7 The values of the recorded shape symmetry in the treatments

4.2.3 Pulp granulation

Appendix (1) shows the results of the analysis of variance for the studied treatments. Significant differences with a P-value of less than 0.05 were found between the treatments. Figure 4-8 illustrates that treatment F1 recorded the highest mean value for the quality trait of interest, reaching 9.50, while treatment F9 recorded the lowest level of crumb firmness at 8.20. The reason for this may be attributed to the ingredients used in the dough preparation or to the temperature and time required for baking.

Additionally, the quality of crumb firmness may be affected by chemical additives such as benzoyl peroxide, potassium bromates, or calcium peroxide in the flour, as previously suggested by Zhang *et al.* (2013), Shanmugavel *et al.* (2020), and Tao *et al.* (2022).

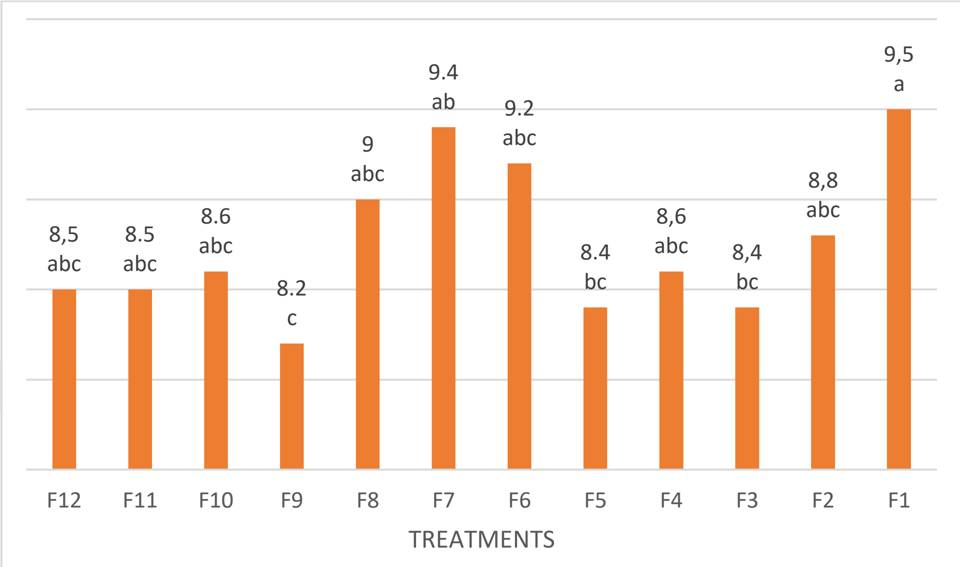

Figure 4.8 The values of the recorded pulp granulation in the treatments

4.2.4 Pulp color

The color of bread crumb depends on the type of bread, its ingredients, and the manufacturing process. Usually, the bread crumb color in commercial bread is white. Annex 1 shows the results of the analysis of variance for the studied treatments. Highly significant differences ($P<0.05$) were found among the treatments under study. Figure 4-9 shows that treatments F3 and F9 recorded the lowest level of the trait at 8.300, while treatments F6 and F7 recorded the highest mean for the trait of bread crumb color at 9.600. The reason for this may be attributed to the type of flour, the manufacturing process, and the temperature used in the baking process. These findings are consistent with those of Fazel-Niari *et al.* (2022). Alternatively, the reason may be due to the effect of chemical additives that enhance the whiteness and remove the natural yellow color of the bread crumb, making it look more attractive and whiter to the eye (Arif *et al.* 2022).

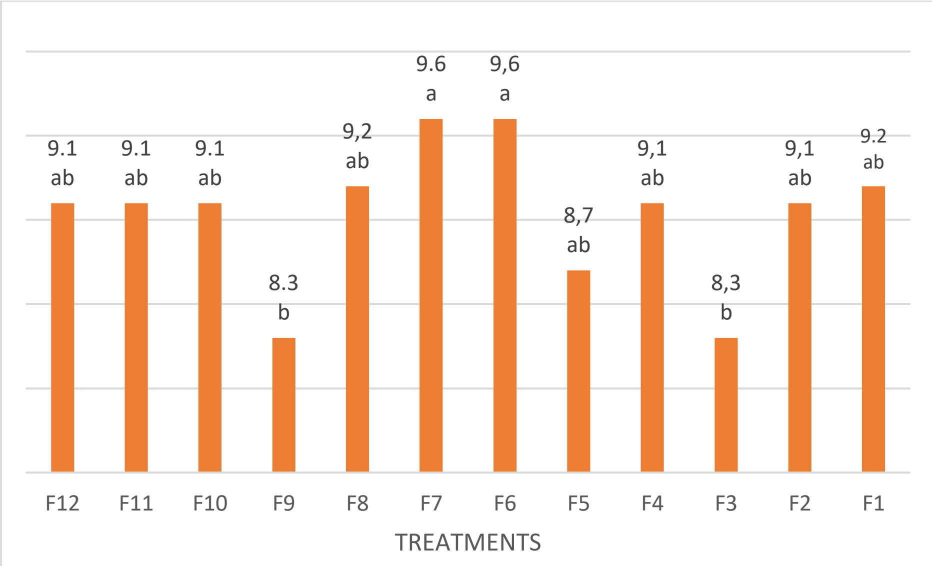

Figure 4.9 The values of the recorded pulp color in the treatments

4.2.5 Pulp tissue

Appendix (1) shows the results of the analysis of variance for the studied treatments, indicating no significant differences at a P<0.05 level of probability between the varieties under study. Figure 4–10 shows the values of the treatments, which are very close together, with treatment F10 recording the highest average value of the trait at 9.20, while treatment F4 recorded the lowest average value of the trait at 7.80. The reason for this may be due to the homogeneity of the flour particles used in the treatments and the decrease in the percentage of damaged starch due to the milling process. This is not consistent with what was mentioned by Boyacioglu and D'Appolonia (1994), as there were no additions, mixing, or reinforcement between the treatments used and other treatments (Arivuchudar *et al.* 2020).

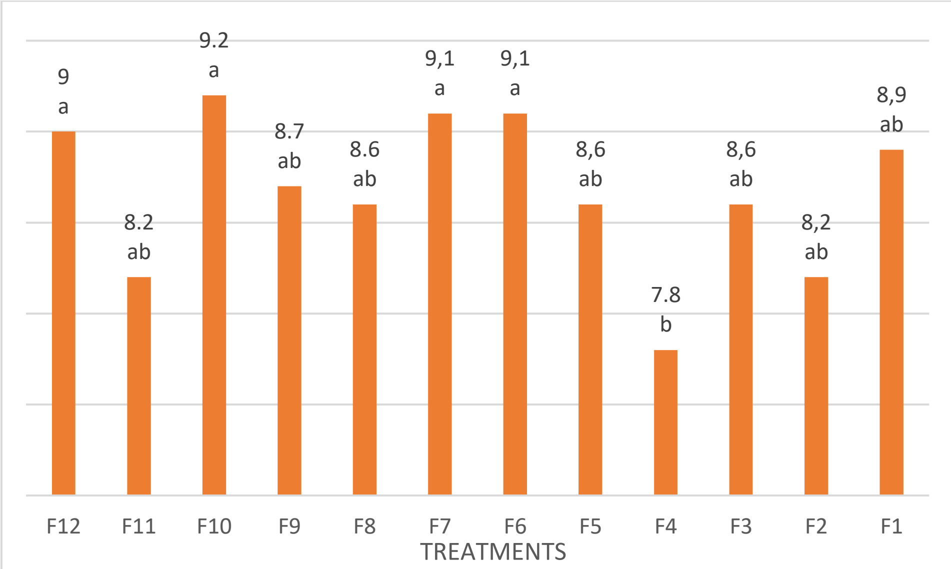

Figure 4.10 The values of the recorded pulp tissue in the treatments

4.2.6 Taste and flavor

Taste and flavor are two important elements in manufactured bread. Taste refers to the individual's sensory experience when consuming bread, while flavor refers to the aromatic ingredients and natural flavors that blend together to add a distinct taste to the bread. Annex 1 shows the results of the variance analysis of the studied treatments. It indicates that there were no significant differences among the treatments under study at a level of $P<0.05$. Figure (4-11) shows the values of the treatments, where treatment F7 recorded the highest average for the characteristic, reaching (18.8), which did not differ significantly from the other treatments, except for treatments F4 and F5, which gave the lowest average, reaching (16.7 and 17), respectively. This may be due to the quality of the ingredients, manufacturing process, and storage. The results are consistent with what was mentioned by Yang *et al.* (2022).

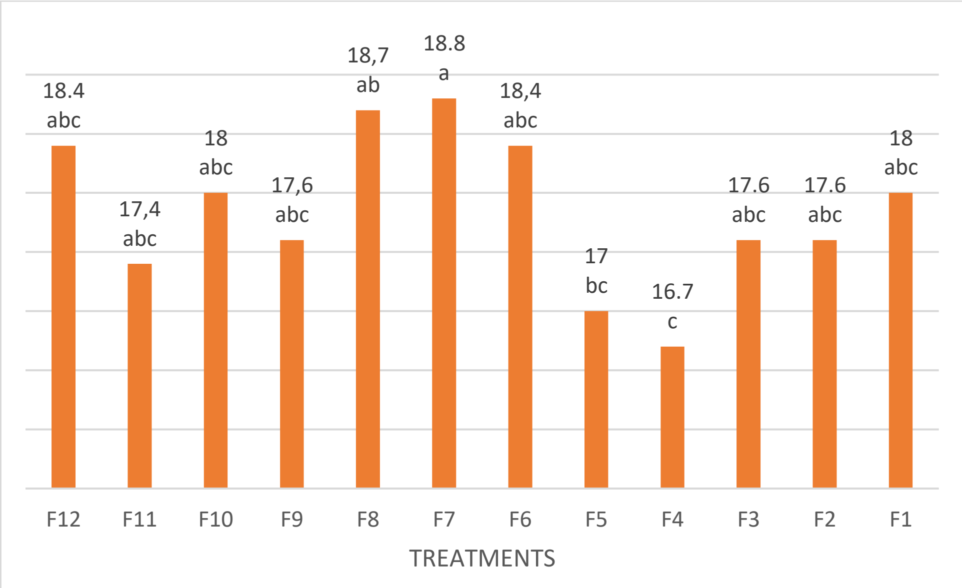

Figure 4.11 The values of the recorded taste and flavor in the treatments

4.3 Chemical additives

Attachment (2) and Table (4-1) showed the results of chemical analysis using the HPLC technique, which revealed the chemical compounds used as additives for reinforcement and trace materials and their concentration in the studied treatments. Treatments F3, F5, F6, and F7 recorded the presence of the compound (Azodi Carbon Amide) with an area of 2.3%, 6.5%, 62.4%, and 17.8%, respectively. While treatments F2, F4, F8, F9, and F10 recorded the presence of the compound (benzoyl peroxid) with an area of (31.9%, 19.9%, 10.5%, 21.8%, and 28.2%), Treatment F1 recorded the presence of the compound (potassium bromate) with an area of 7.4%, and treatment F12 recorded the presence of the compound (calcium peroxide) with an area of 41.4%. However, treatment F11 did not record the presence of any chemical compound. The presence of these bleaching and flour quality-enhancing agents has very negative effects on human health, and they are banned for use within the Iraqi specifications for imported flour (Olofinnade *et al.* 2020).

Table 4.1 Chemical analysis results using HPLC technique

Treatment	Reten. Time(min)	Area (mV.s)	Height (mV)	Area (%)	Height (%)	W05 (min)	Compound Name
F1	4.244	120.155	19.336	7.4	31.5	0.12	Potassium Bromate
F2	6.648	341.535	10.554	31.9	23.3	0.34	Benzoyl Peroxid
F3	1.660	58.981	3.916	2.3	4.3	0.34	Azodi Carbon Amide
F4	7.648	368.100	74.033	19.9	42.6	0.09	Benzoyl Peroxid
F5	1.500	60.421	10.942	6.5	59.4	0.12	Azodi Carbon Amide
F6	1.508	244.702	6.476	62.4	27.0	0.13	Azodi Carbon Amide
F7	1.684	170.690	21.086	17.8	38.4	0.13	Azodi Carbon Amide
F8	7.548	413.647	17.084	10.5	8.4	0.47	Benzoyl Peroxid
F9	7.676	324.135	80.447	21.8	59.4	0.07	Benzoyl Peroxid
F10	7.604	382.035	50.080	28.2	55.4	0.12	Benzoyl Peroxid
F11	-	-	-	-	-	-	-
F12	2.824	179.331	20.894	41.4	83.3	0.16	Calcium Peroxide

5. CONCLUSIONS

Based on the chemical tests conducted on the study treatments, we conclude the following:

1. Imported flour varieties outperformed local flour in all rheological and sensory attributes, yielding high sensory evaluation results.

2. Imported flour varieties contain additives and bleaching agents, some of which are prohibited substances according to Iraqi standards.

3. These additives have enhanced the quality and manufacturing properties of the imported flour at the expense of local flour, leading consumers to focus on using imported varieties.

4. White imported varieties showed superior performance compared to the local type in terms of color.

6. RECOMMENDATIONS

1. The added compounds (potassium bromate, benzoyl peroxide, and azodi carbon dioxide) are prohibited substances in Iraq. We recommend not using them in imported flour from Iraq and in locally produced flour.

2. Enhance the quality control measures at the border points and emphasize the examination of these additives (potassium bromate, benzoyl peroxide, and azodi carbon dioxide).

3. Prepare the necessary materials and precise laboratory equipment at the border points to examine imported flour.

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APPENDICES

APPENDIX 1.

ANOVA Table						
		Sum of Squares	df	Mean Square	F	Sig.
Flour color	Between Groups	80.934	11	7.358	10.615	.000
	Within Groups	16.635	24	.693		
	Total	97.570	35			
protein	Between Groups	27.808	11	2.528	21.982	.000
	Within Groups	2.760	24	.115		
	Total	30.568	35			
Humidity	Between Groups	14.216	11	1.292	12.085	.000
	Within Groups	2.567	24	.107		
	Total	16.783	35			
permeability	Between Groups	2893.883	11	263.080	266.015	.000
	Within Groups	23.735	24	.989		
	Total	2917.618	35			
Ash	Between Groups	3.349	11	.304	2082.091	.000
	Within Groups	.004	24	.000		
	Total	3.352	35			

Table of averages and the experimental error for the averages of the chemical content of the experiments

Transactions	Flour color	Protein	Humidity	Permeability	Ash
	Mean & Std. Error	Mean & Std. Error	Mean & Std. Error	Mean & Std. Error	Mean & Std. Error
F1	91.20 ± .60 ab	± .12 f11.30	12.97± .09 a	± .88 a98.33	.65 ± .0088 g
F2	90.00 ± .58 ab	.22 b ± 13.37	11.43 ± .23 cd	99.00 ± .58 a	.77 ± .0044 d
F3	91.00 ± 1.00 ab	± .09 b 13.03	12.00 ± .11 bc	99.66± .66 a	.91± .0039 b
F4	90.30 ± .35 ab	12.27± .23 c	11.57 ± .29 bc	98.33 ± .88 a	.74 ± .0028 e
F5	90.63 ± .52 ab	± .28 f11.23	± .19 b12.13	99.83± .60 a	.71 ± .0028 f
F6	90.43 ± .45 ab	± .24 ef11.47	10.97 ± .09 d	98.20 ± .47 a	.72± .0023 ef
F7	89.73 ± .15 ab	±.12 a14.07	13.06 ± .18 a	98.30 ± .21 a	.82 ± .0026 c
F8	91.40 ± .21 a	± .26 def11.57	11.90 ± .06 bc	99.33 ±.49 a	.58± .0021 h
F9	90.34 ± .18 ab	± .19 cd12.13	11.67 ± .28 bc	98.84 ± .33 a	.72 ± .0028 ef
F10	90.53 ± .35 ab	± .23 c 12.37	12.83 ± .08 a	98.10± .55 a	.65 ± .0060 g
F11	85.37 ± .38 b	± .15 b13.30	11.73 ± .18 bc	66.47 ± .35 b	1.80± .0173 a
F12	90.30 ± .32 ab	12.00 ± .06 cde	11.57 ± .70 bc	99.30 ± .46 a	.82 ± .0097 c

ANOVA Table

		Sum of Squares	df	Mean Square	F	Sig.
Shell color	Between Groups	29.818	10	2.982	3.157	.002
	Within Groups	93.500	99	.944		
	Total	123.318	109			

Shape symmetry	Between Groups	8.473	10	.847	1.545	.135
	Within Groups	54.300	99	.548		
	Total	62.773	109			
Pulp granulation	Between Groups	18.964	10	1.896	1.844	.063
	Within Groups	101.800	99	1.028		
	Total	120.764	109			
Pulp color	Between Groups	19.018	10	1.902	2.481	.011
	Within Groups	75.900	99	.767		
	Total	94.918	109			
Pulp tissue	Between Groups	19.055	10	1.905	1.541	.136
	Within Groups	122.400	99	1.236		
	Total	141.455	109			
Taste and flavor	Between Groups	43.800	10	4.380	1.496	.152
	Within Groups	289.800	99	2.927		
	Total	333.600	109			

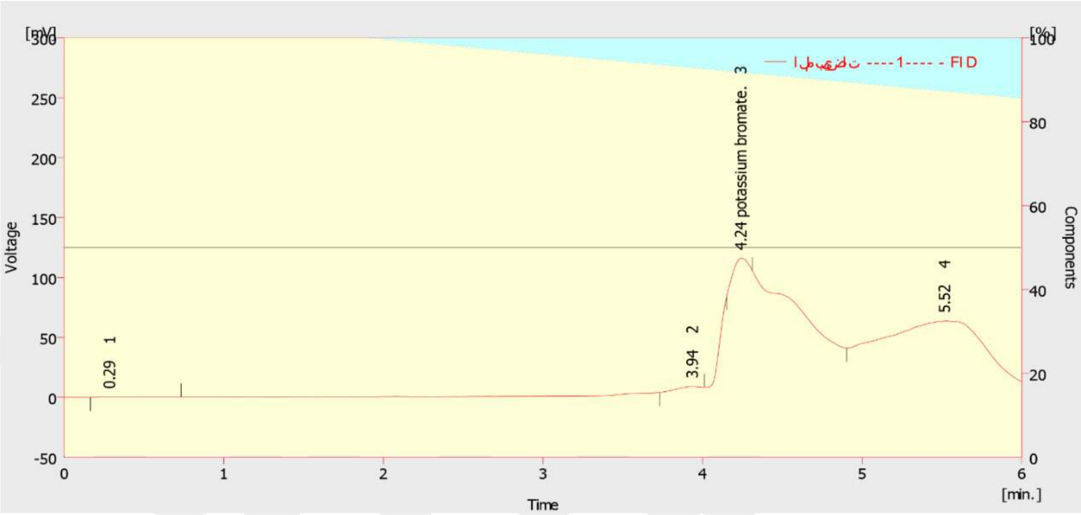
Table of means and experimental error for the sensory evaluation averages of the experiments

Transactions	**Shell color**	**Shape symmetry**	**pulp granulation**	**pulp color**	**pulp tissue**	**Taste and flavor**
	Mean & Std. Error	Mean & Std. Error	Mean & Std. Error	Mean & Std. Error	Mean & Std. Error	Mean & Std. Error
F1	8.600 ± 0.31 bcd	± 0.20 abc3.800	9.500± 0.22 a	9.200 ± 0.29 ab	8.900 ± 0.43 ab	18.000 ± 0.65 abc
F2	9.200 ±0.33 abc	0.22a ± 4.500	8.800 ± 0.20 abc	9.100 ± 0.31 ab	8.200 ± 0.36 ab	17.600 ± 0.54 abc

F3	9.200± 0.33 abc	± 0.25abc4.200	8.400 ± 0.27 bc	8.300 ± 0.26 b	8.600 ± 0.48 ab	17.600 ± 0.69 abc
F4	8.500 ±0.31 bcd	± 0.29 abc3.800	8.600 ± 0.31 abc	9.100 ± 0.23 ab	7.800 ± 0.29 b	16.700 ± 0.75 c
F5	8.400 ± 0.34 cd	± 0.20 abc3.800	8.400 ± 0.48 bc	8.700 ± 0.30 ab	8.600 ± 0.40 ab	17.000 ± 0.61 bc
F6	7.700 ± 0.30 c	± 0.20 abc4.200	9.200 ± 0.33 abc	9.600 ± 0.31 a	9.100 ± 0.28 a	18.400 ± 0.54 abc
F7	9.800 ± 0.13 a	± 0.26 bc3.700	9.400 ± 0.22 ab	9.600 ± 0.16 a	9.100 ± 0.31 a	18.800 ± 29 a
F8	9.100 ± 0.31abc	± 0.21abc4.000	9.000 ± 0.37 abc	9.200 ±0.33 ab	8.600 ± 0.31 ab	18.700 ± 0.30 ab
F9	8.800 ± 0.29 bc	± 0.17 c3.500	8.200 ± 0.33 c	8.300 ± 0.30 b	8.700 ± 0.26 ab	17.600 ± 37 abc
F10	8.600 ± 0.34 bcd	± 0.29 abc4.200	8.600 ± 0.34 abc	9.100 ± 0.28 ab	9.200 ± 0.29 a	18.000 ± 47 abc
F11	8.600 ± 0.34bcd	± 0.25 abc3.800	8.500 ± 0.37 abc	9.100 ± 0.23 ab	8.200 ± 0.39 ab	17.400 ± 0.52 abc
F12	9.400 ± 0.22 ab	4.300 ± 0.21 ab	8.500 ± 0.17 abc	9.100 ± 0.35 ab	9.000 ± 0.33 a	18.400 ± 0.31 abc

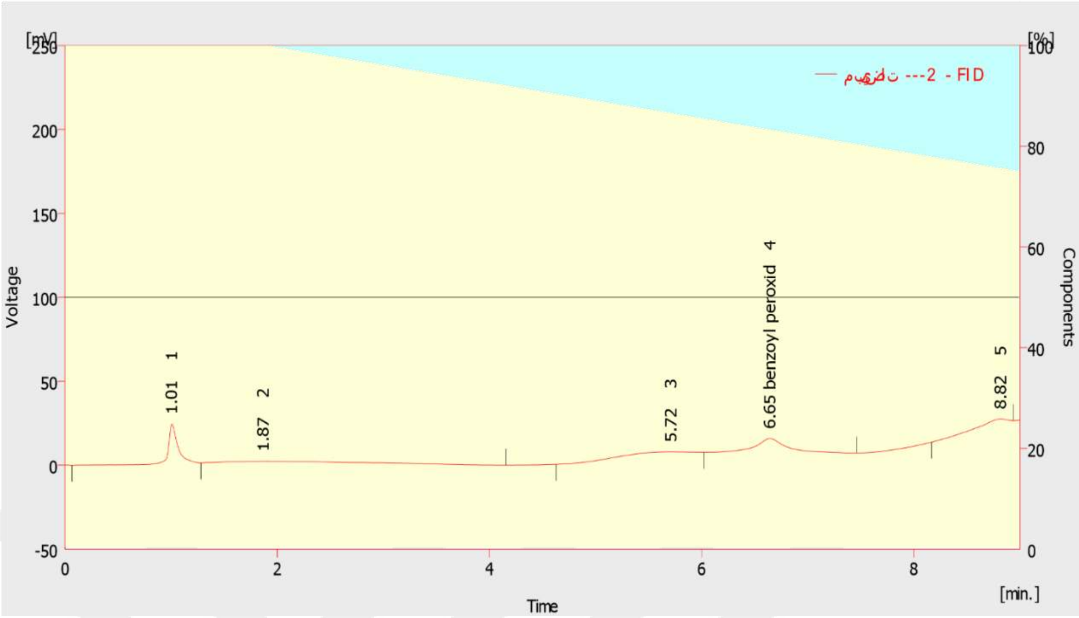
APPENDIX 2

Analysis of F1 with HPLC



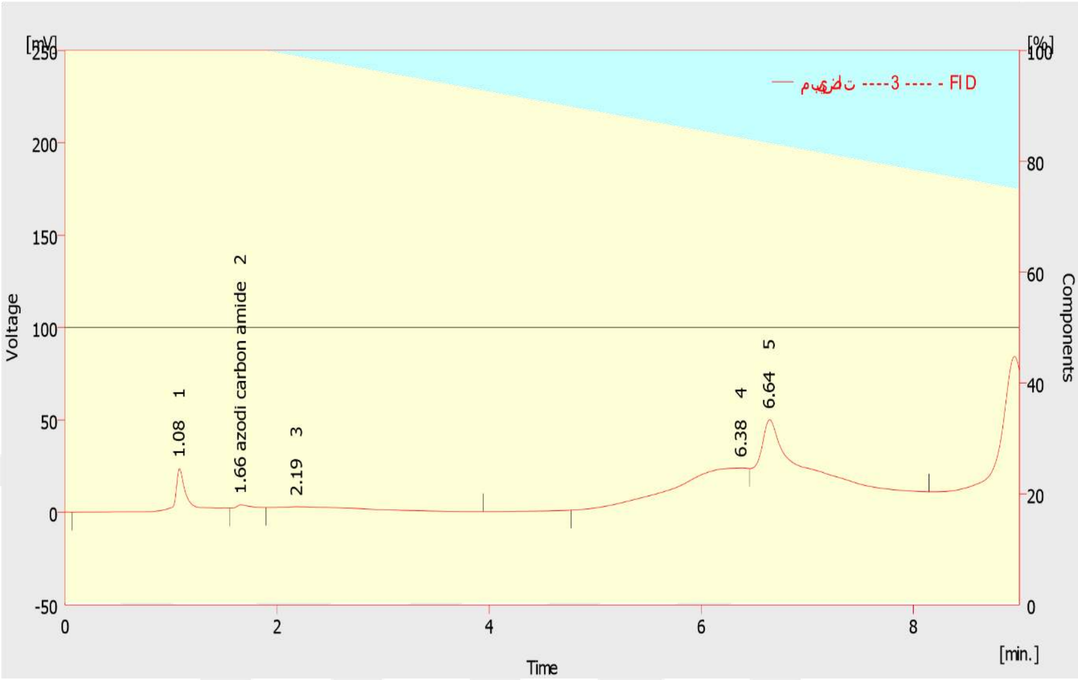
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	0.288	6.515	0.402	0.4	0.7	0.50	
2	3.940	16.956	1.891	1.1	3.1	0.14	
3	4.244	120.155	19.336	7.4	31.5	0.12	potassium bromates.
4	5.520	1469.644	39.661	91.1	64.7	0.79	
	Total	1613.269	61.290	100.0	100.0		

Analysis of F2 with HPLC



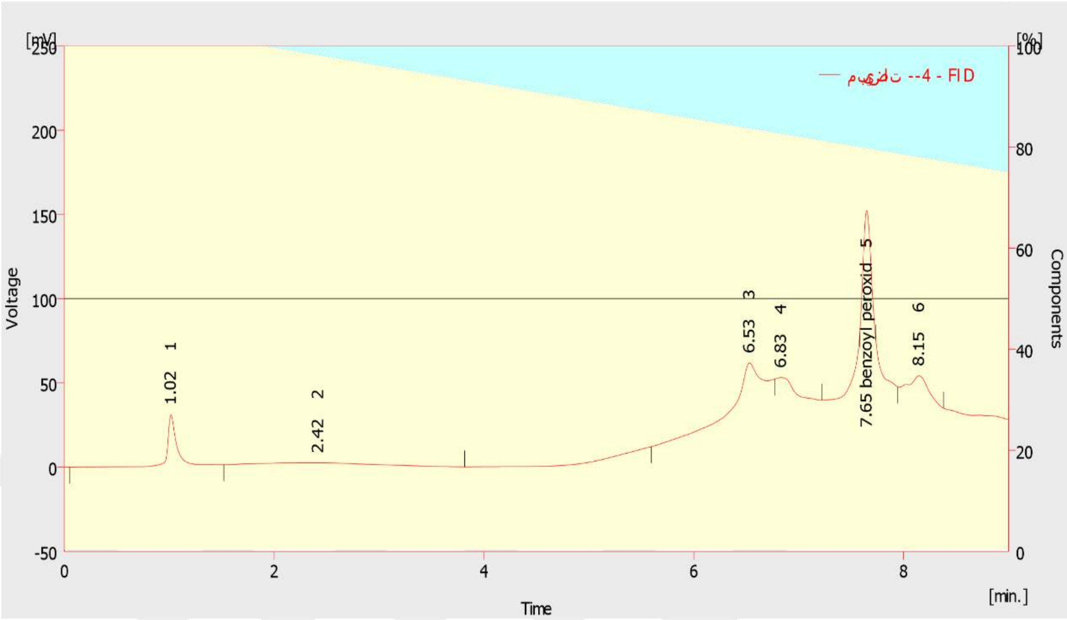
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.008	178.784	24.524	16.7	54.2	0.08	
2	1.872	240.663	2.408	22.5	5.3	1.90	
3	5.716	254.461	4.876	23.8	10.8	0.77	
4	6.648	341.535	10.554	31.9	23.3	0.34	benzoyl peroxid
5	8.824	55.568	2.913	5.2	6.4	0.21	
	Total	1071.011	45.275	100.0	100.0		

Analysis of F3 with   HPLC



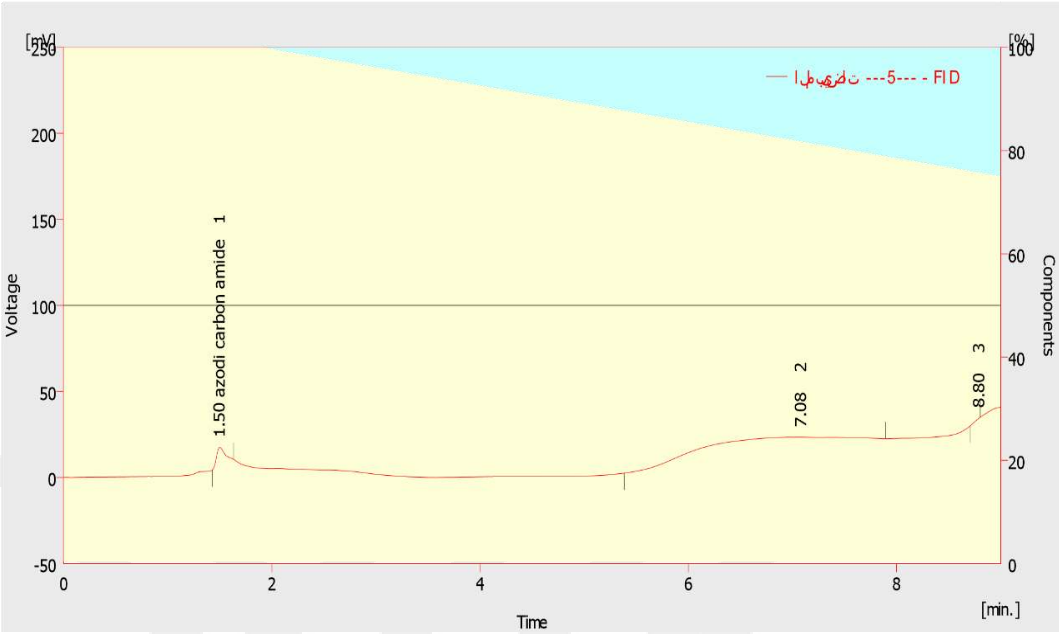
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.080	206.359	23.592	8.1	25.7	0.09	
2	1.660	58.981	3.916	2.3	4.3	0.34	azodi carbon amide
3	2.188	169.838	2.796	6.6	3.0	1.01	
4	6.380	856.036	18.082	33.4	19.7	0.64	
5	6.644	1269.456	43.460	49.6	47.3	0.30	
	Total	2560.671	91.846	100.0	100.0		

Analysis of F4 with HPLC



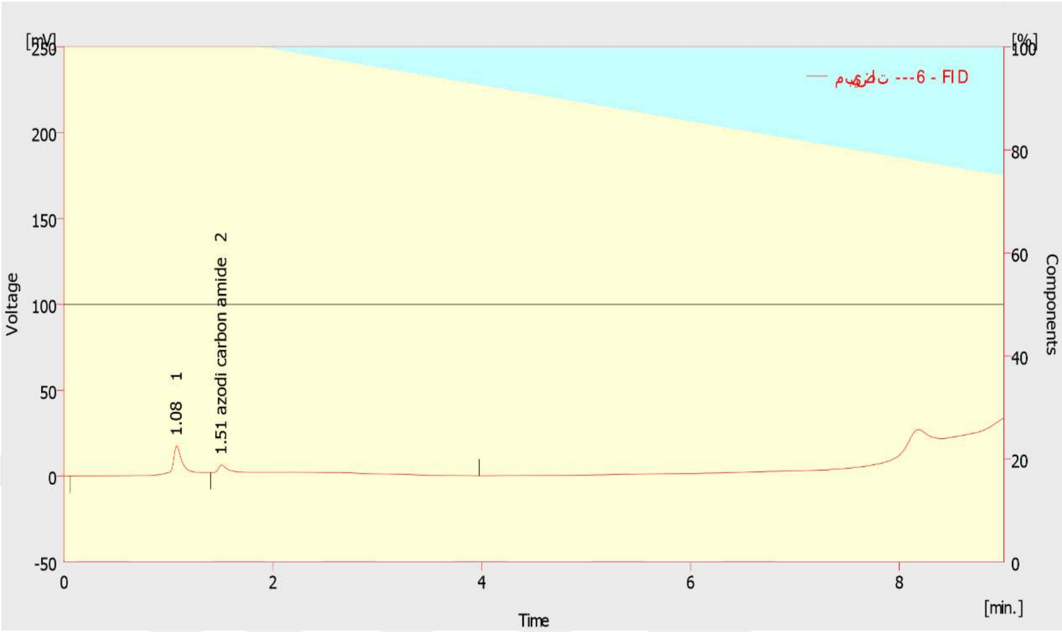
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.016	217.564	31.003	11.8	17.8	0.08	
2	2.420	214.909	2.530	11.6	1.5	1.54	
3	6.532	649.805	33.829	35.2	19.5	0.32	
4	6.832	257.263	20.029	13.9	11.5	0.22	
5	7.648	368.100	74.033	19.9	42.6	0.09	benzoyl peroxid
6	8.148	140.299	12.458	7.6	7.2	0.24	
	Total	1847.940	173.880	100.0	100.0		

Analysis of F5 with HPLC



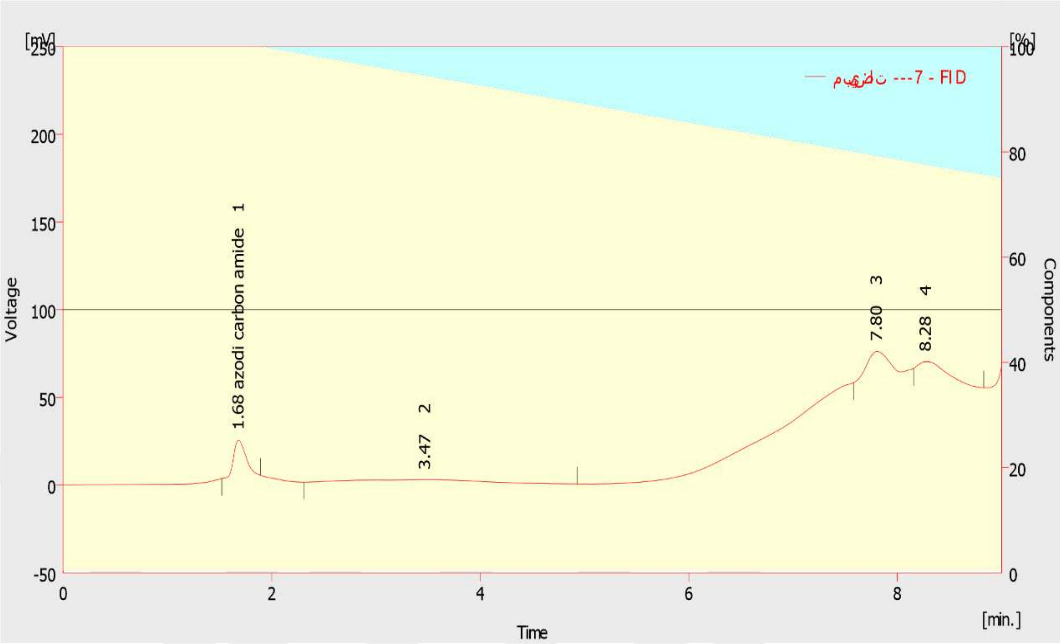
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.500	60.421	10.942	6.5	59.4	0.12	azodi carbon amide
2	7.084	869.468	7.396	93.5	40.2	1.59	
3	8.796	0.009	0.070	0.0	0.4	0.01	
	Total	929.898	18.408	100.0	100.0		

Analysis of F6 with HPLC



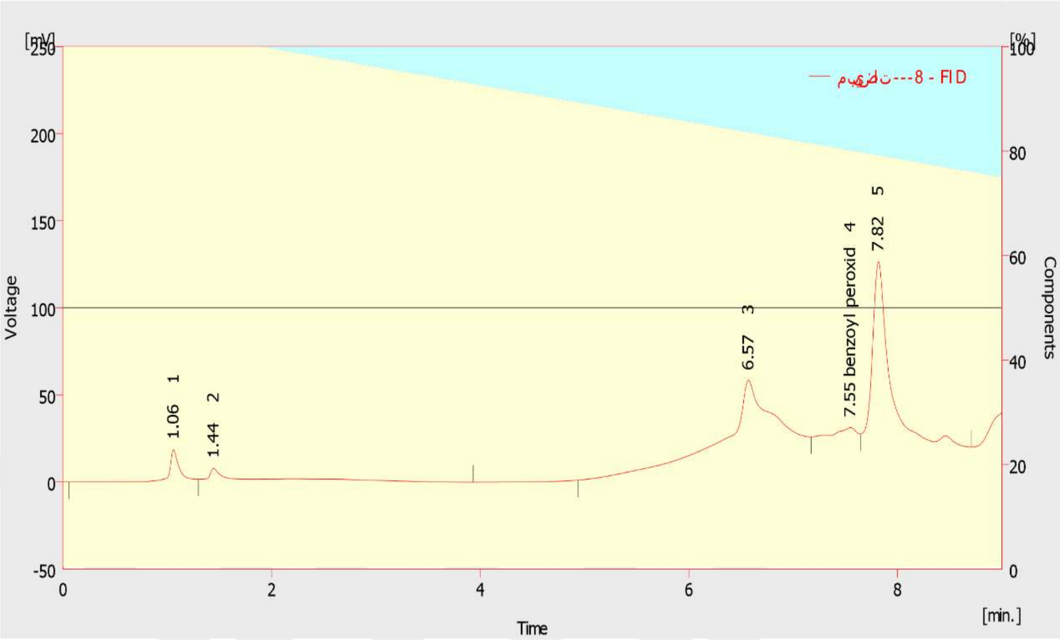
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.080	147.625	17.537	37.6	73.0	0.09	
2	1.508	244.702	6.476	62.4	27.0	0.13	azodi carbon amide
	Total	392.327	24.012	100.0	100.0		

Analysis of F7 with HPLC



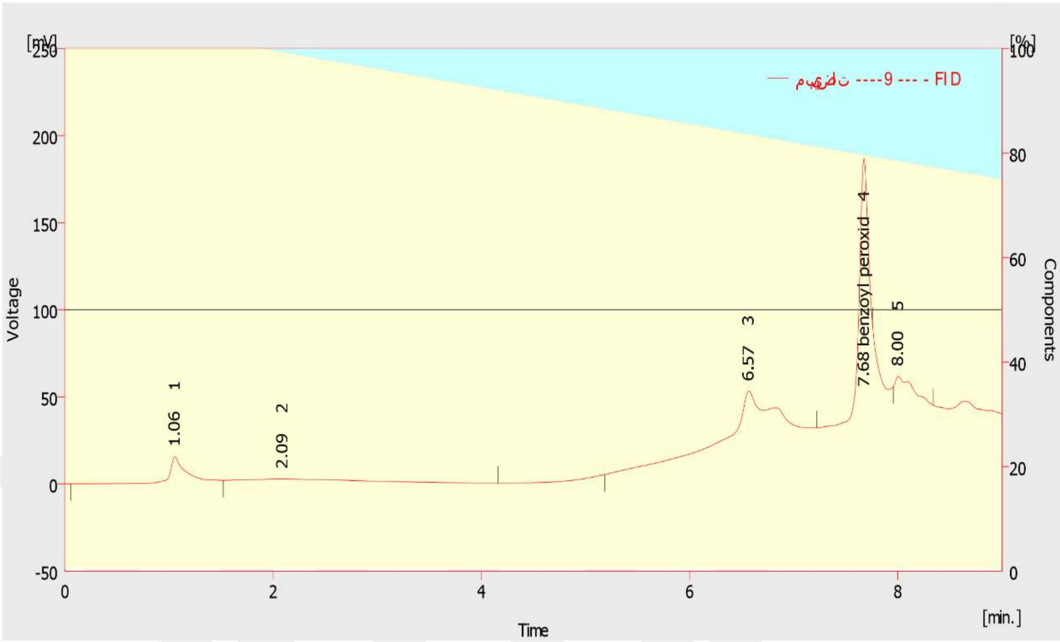
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.684	170.690	21.086	17.8	38.4	0.13	azodi carbon amide
2	3.472	148.175	1.827	15.5	3.3	1.47	
3	7.804	357.597	18.419	37.4	33.5	0.28	
4	8.276	279.834	13.587	29.3	24.7	0.33	
	Total	956.295	54.920	100.0	100.0		

Analysis of F8 with HPLC



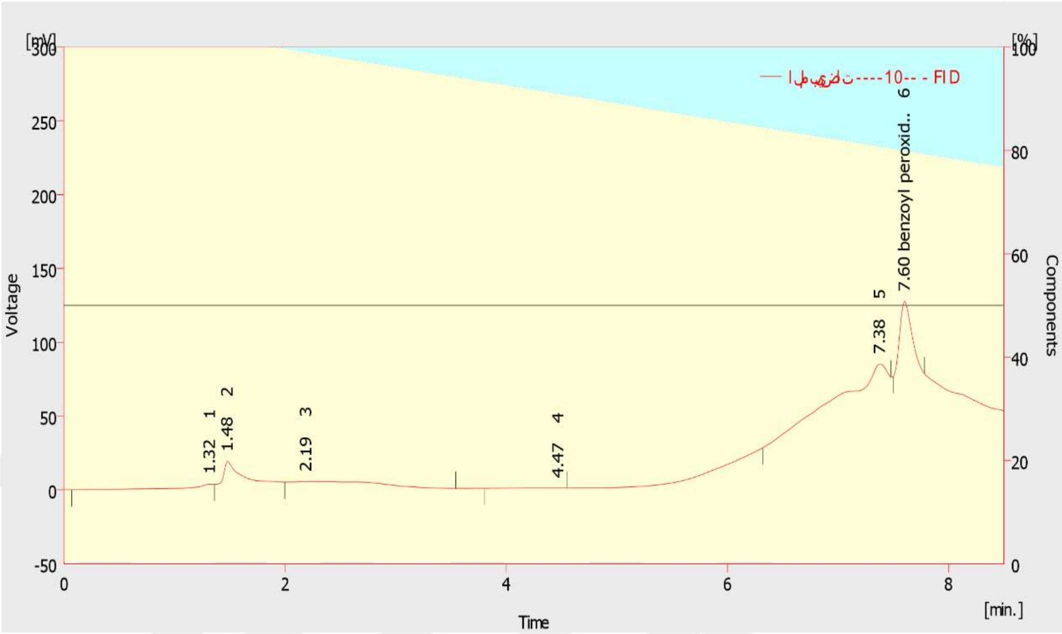
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.060	130.643	18.592	3.3	9.1	0.08	
2	1.444	224.763	7.902	5.7	3.9	0.11	
3	6.572	1722.520	49.158	43.7	24.1	0.42	
4	7.548	413.647	17.084	10.5	8.4	0.47	benzoyl peroxid
5	7.816	1447.346	110.998	36.7	54.5	0.15	
	Total	3938.919	203.733	100.0	100.0		

Analysis of F9 with HPLC



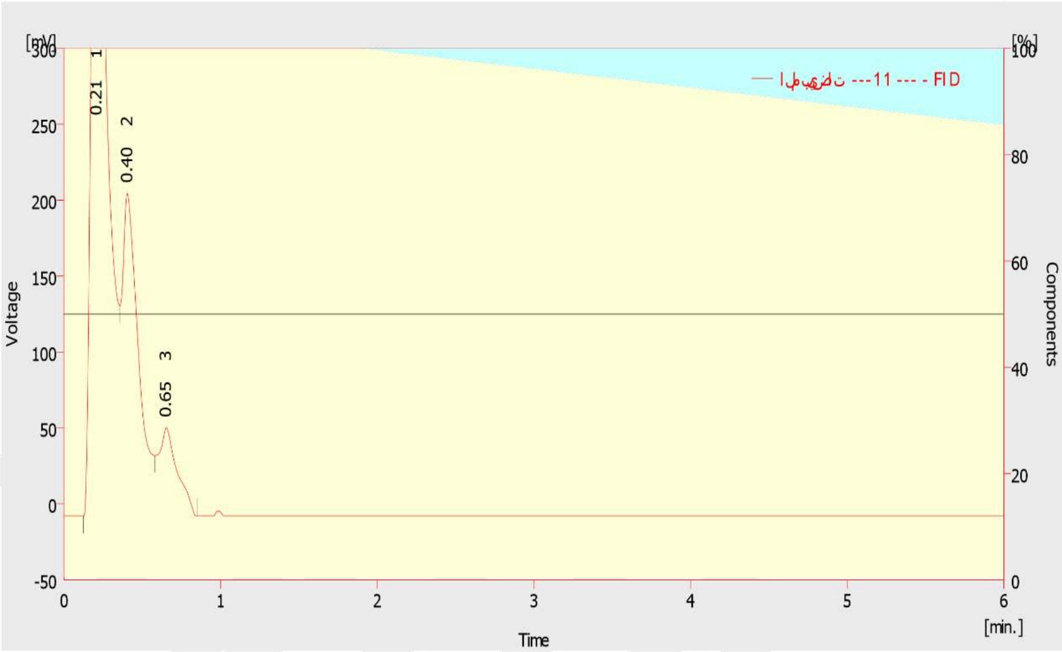
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.056	188.383	15.500	12.7	11.5	0.13	
2	2.092	220.029	2.646	14.8	2.0	1.46	
3	6.568	674.487	29.654	45.3	21.9	0.41	
4	7.676	324.135	80.447	21.8	59.4	0.07	benzoyl peroxid
5	8.004	80.987	7.123	5.4	5.3	0.09	
	Total	1488.020	135.370	100.0	100.0		

Analysis of F10 with HPLC



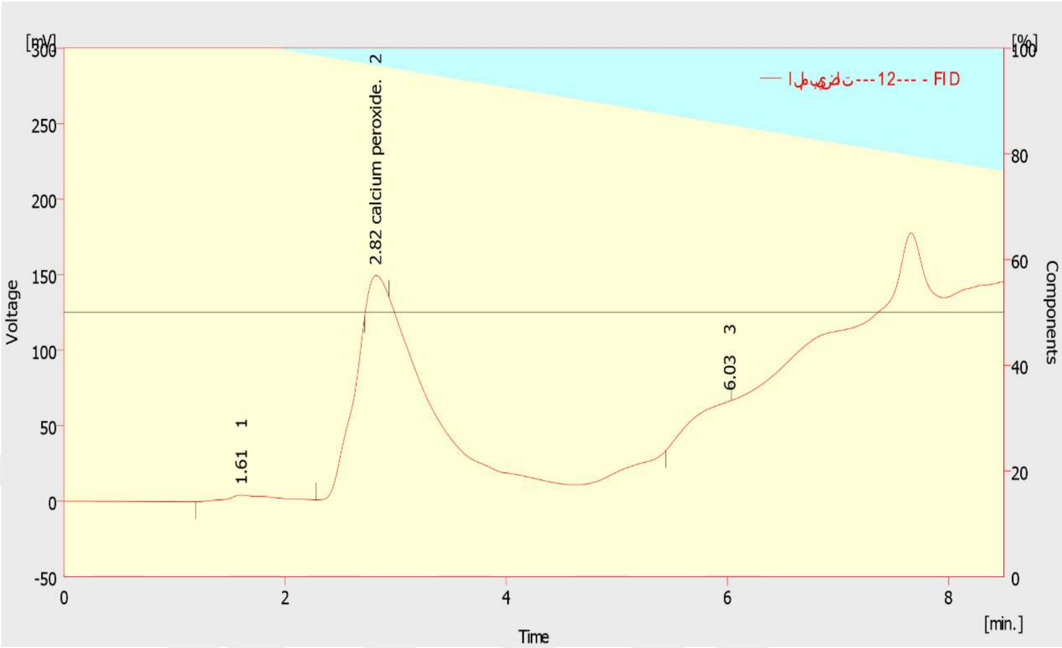
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.320	59.489	3.543	4.4	3.9	0.13	
2	1.480	296.003	18.712	21.8	20.7	0.17	
3	2.192	290.286	4.994	21.4	5.5	0.99	
4	4.472	3.506	0.074	0.3	0.1	0.13	
5	7.380	324.669	12.918	23.9	14.3	0.14	
6	7.604	382.035	50.080	28.2	55.4	0.12	benzoyl peroxid..
	Total	1355.988	90.320	100.0	100.0		

Analysis of F11 with HPLC



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	0.208	3967.379	580.559	25.0	39.1	0.10	
2	0.404	1580.677	212.485	10.0	14.3	0.12	
3	0.652	495.158	58.080	3.1	3.9	0.14	
4	19.908	3196.780	161.688	20.1	10.9	0.35	
5	20.276	1678.704	119.516	10.6	8.1	0.27	
6	20.492	1577.910	125.282	9.9	8.4	0.22	
7	20.664	2298.168	113.754	14.5	7.7	0.39	
8	21.068	930.178	75.261	5.9	5.1	0.20	
9	21.468	50.512	14.241	0.3	1.0	0.06	
10	21.660	101.248	23.142	0.6	1.6	0.08	

Analysis of F12 with HPLC



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.608	103.217	3.954	23.8	15.8	0.44	
2	2.824	179.331	20.894	41.4	83.3	0.16	calcium peroxide.
3	6.028	150.785	0.246	34.8	1.0	0.02	
	Total	433.334	25.094	100.0	100.0		

CURRICULUM VITAE

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Work Experience

Year	**Institution**	**Position**
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Academic Activities