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Quantitative Analysis of Potassium Bromate, Potassium Iodate and Alloxan in White Flour by using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR)

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Declaration

I affirm that this thesis, submitted for the master's degree in the Applied and Industrial Technology Program, is the product of my research, except where explicitly cited, and that this thesis (or any portion thereof) has not been submitted for a higher degree to any other university or institution.

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Abstract

Wheat is a critical cereal crop around the world, and it is a major part of our daily diet as a main food source. There are different kinds of wheat, such as Triticum aestivum. It is the main source of flour for making bread. Although whole wheat grains are wealthy in beneficial elements, the flour made from this wheat lacks desirable rheological features; therefore, it is important to add some additives to improve its characteristics. Unfortunately, chemical additives used in wheat flour, particularly oxidizing and bleaching agents, have raised increasing concerns regarding food safety and human health. This study aims to determine the quantity of potassium bromate (PB), alloxan, and potassium iodate (PI) in white flour using Attenuated Total Reflectance–Fourier Transform Infrared (ATR-FTIR) spectroscopy. Standard flour mixtures containing different concentrations of each additive were prepared and analyzed to identify characteristic absorption bands. Calibration curves were developed utilizing specific infrared (IR) absorbance ratios, facilitating the quantitative assessment of each additive in wheat matrices by using several equations, including the following:

PB (ppm) = -86.717**r** + 115.66 $R^2 = 0.9894$.

Alloxan (ppm) = 49.144**r** - 21.165 $R^2 = 0.9878$.

PI (ppm) = -90.911**r** + 291.53 $R^2 = 0.9867$.

The variable r represents the absorbance ratio at certain wavenumbers, while R^2 is coefficient of determination.

The validated ATR-FTIR method was utilized to examine various commercial white flour samples obtained from the Palestinian market. The results demonstrated excellent linearity, sensitivity, and reproducibility for all investigated additives. Moreover, they revealed the presence of varying concentrations of PB, PI, and alloxan in some commercial flour samples, which could be a public health concern. The proposed method offers a rapid, minimal sample preparation, cost-effective, and non-destructive alternative to conventional analytical techniques and can be effectively applied for routine monitoring and quantitative assessment of flour additives to support food safety, quality control, and regulatory compliance.

Keywords: additives, flour, potassium bromate, alloxan, potassium iodate, ATR-FTIR, calibration curves, analytical method.

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Table of Abbreviations

Name	Abbreviations
Acceptable Daily Intake	ADI
Analog-to-Digital	A/D
Attenuated Total Reflectance-Fourier Transform Infrared	ATR-FTIR
Azodicarbonamide	ADA
Bacterial Nanocellulose	BNC
Calcium Stearoyl Lactylate	CSL
CarboxymethylCellulose	CMC
Carrageenans	CA
Cistus Incanus L	CI
Coefficient of Determination	R^2
Coeliac Disease	CD
Dermatitis Herpetiformis	DH
Diacetyl Tartaric Acid Esters of Monoglycerides	DATEM
Distilled Monoglycerides	DMG
Ethoxylated Monoglycerides	EMG
European Food Safety Authority	EFSA
European Union	EU
Fingerprint	FP
Food and Agriculture Administration	FAO
Food and Drug Administration	FDA
Functional Group	F/G
Generally Recognized as Safe	GRAS
Glucose Oxidase	GOX
Gluten Ataxia	GA
Guar Gum	GG
High-Performance Liquid Chromatography	HPLC
Hydroxypropylmethylcellulose	HPMC
Infrared	IR
International Agency for Research on Cancer	IARC
International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use	ICH
International Numbering System	INS
Joint FAO/WHO Expert Committee on Food Additives	JECFA
Laccase	LAC
Limit of Detection	LOD

Limit of Quantification	LOQ
Locust Bean Gum	LBG
Mono- and Diglycerides	MDG
National Agency for Food and Drug Administration and Control	NAFDAC
Non-Coeliac Gluten Sensitivity	NCGS
Polysorbates	PS
Post Column Reagent	PCR
Potassium Bromate	PB
Potassium Iodate	PI
Reactive Oxygen Species	ROS
Relative Standard Deviation	RSD
Sodium Stearoyl Lactylate	SSL
Standard Deviation	SD
Stearoyl Lactylate	SL
Thin Layer Chromatography	TLC
Transglutaminase	T Gase
Ultra- High Performance Liquid Chromatography- Tndem Mass Spectrometry	UHPLC MS/MS
UltraViolet	UV
United Kingdom	UK
United States of America	USA
Unregulated Organic Chemicals	UOCs
World Health Organization	WHO
Xanthan gum	XG

Chapter One

1. Introduction

1.1 Background

Wheat is a crucial cereal crop of significant global importance, with the United States (USA), India, and China as the leading producers (Tian et al., 2022b). Wheat is grown in various climates and soils, showcasing its flexibility to different agricultural situations (Zubko et al., 2022). It has profoundly impacted human civilization and remains a vital element of our everyday existence as a key food source (Turky et al., 2023). The various wheat cultivars, including 14 species globally, are categorized based on their chromosomal count. The categories include diploid wheat with 14 chromosomes, such as einkorn wheat; tetraploid wheat with 28 chromosomes, represented by durum and emmer wheat; and hexaploid wheat with 42 chromosomes, exemplified by common or bread wheat (Wrigley, 2009). Each kind of wheat has unique characteristics and is suitable for specific uses. Triticum aestivum, often known as common wheat, is the most extensively cultivated species worldwide and serves as the primary source of flour for bread production. For millennia, humanity has extensively consumed wheat, with more than half of the global population depending on it as a principal source of protein and carbohydrates. Wheat has several purposes, chiefly in the production of flour for bread, pasta, and assorted baked goods. It serves as a feed grain for livestock and as a precursor for ethanol manufacturing. Whole wheat grain is an outstanding source of complex carbohydrates, dietary fiber, and many vitamins and minerals, making it an essential food crop for human consumption (Mathews and Chu, 2021). Wheat flour is the primary form of wheat grain processing, as most flour-based products are derived from it (Gómez et al., 2020). The wheat kernel comprises three primary components: germ, bran, and endosperm. The endosperm is the principal component, primarily made up of starch granules inside a proteinaceous matrix, comprising 81–84% of the grain. The germ consists of the embryo and scutellum, making up 2–3% of the grain. Bran, constituting 14–16% of the grain, encompasses all outer layers, including

the aleurone layer, which is frequently removed with the other bran layers during milling (Inamdar and Suresh, 2014).

The milling of wheat is a crucial process in producing numerous flour types, which are excellent raw materials for making different cereal goods. The milling of wheat flour enables the extraction of the primary component of the wheat grain, known as the starchy endosperm, which contains vital technological functioning elements. The separation is achieved by extracting the bran and germ from the endosperm (Carcea et al., 2022). The milling processes alter the makeup of all constituents in the flour, thereby affecting its nutritional quality. Furthermore, they affect particle size, which is crucial in determining the functional and nutritional properties of the flour (Cappelli and Cini, 2021). Wheat milling consists of several stages: cleaning, conditioning, grinding, and sifting. Each stage affects the yield and properties of the flour. Multiple milling processes utilize diverse grinding and sifting equipment. Roller milling is the primary method for creating refined wheat flour. This approach entails the gradual separation of endosperm from the bran and germ, followed by a methodical reduction in the size of the endosperm particles, accompanied by filtering between grinding stages (Cappelli et al., 2020b). However, in some countries, stone milling is utilized (Carcea et al., 2020). The results of milling are greatly affected by the properties of wheat grain, proper milling preparation, and the grinding technique employed (Kalitsis et al., 2021). Recent advancements in quick grain testing, cleaning, and milling processes have enhanced milling efficiency and flour quality. Furthermore, recent innovations have advanced and revolutionized the ultra-fine grinding technology for wheat flour and its derivatives. This has led to the manufacturing of many varieties of flour and bran powders, each possessing distinct features and applications (Dziki, 2023; Lai et al., 2023; Lin et al., 2021).

1.2 Triticum Aestivum L (Hexaploid Wheat)

Hexaploidy wheat (Triticum aestivum L), commonly referred to as bread wheat, is among the most extensively employed varieties, especially esteemed for the manufacturing of bread. It is cultivated in moist circumstances that yield delicate cereals, containing 8–10% protein and minimal gluten content. Gluten comprises gliadin, a prolamin, and glutenin, a glutelin. During ingestion, the prolamins, specifically gliadin and glutenin, which form gluten, must be broken down and digested in the small intestine's lumen; nevertheless, their intricate peptide structures present a challenge for human digestion owing to their elevated proline and glutamine content. These prolamins precipitate several illnesses, including coeliac disease (CD), non-coeliac gluten sensitivity (NCGS), gluten ataxia (GA), and dermatitis herpetiformis (DH) (Khoury et al., 2018; Niland and Cash, 2018). The primary application of wheat flour is in the manufacture of bread, cakes, crackers, biscuits, pastries, and assorted domestic flours. The types of wheat employed consist of wheat germ oil, bran, wheat germ flour, whole grain flour, wheatgrass, and whole grains. The industry has established precise methods for starch extraction from maize and wheat. Utilizing a low-water input centrifugation technique, contemporary manufacturing plants may produce 40 to 50 tons of flour an hour. Starch from wheat is currently employed in various items, featuring culinary

additions like binders in soups and sauces, sweeteners in beverages, and moisture-retaining agents in baked products. Moreover, textural agents are employed in diverse dairy products, bioplastics, green chemistry (fermentation), ethanol generation, paper production, emulsifiers, infant nutrition, energy beverages, piglet starter feed, aquaculture feed pellets, animal feed (milk powder), and additional applications (Igrejas and Branlard, 2020). Gluten is a high-quality secondary product generated by the industry of wheat starch. Gluten is currently the most cost-effective "green protein" accessible for the feed and food industries, owing to the heightened global supply of the starch of wheat. Gluten powder has been integrated with flour to improve its technical and rheological characteristics, thereby meeting baking industry standards. Additionally, wheat functions as an animal feed grain, a utilization significantly influenced by price fluctuations relative to other commodities. Therefore, when meteorological conditions affect harvests and render excess grain unfit for human consumption, we designate it for livestock feed. Substandard grain is employed in the manufacture of alcohol, adhesives, paper additives, soaps, rubbers, cosmetics, and varnishes, leading to heightened demand and production (Ammar et al., 2023).

1.3 Food Additives

Additives are substances introduced in minimal quantities to improve the flavor, texture, appearance, or shelf life of a product (Pressman et al., 2017). The European Union (EU) has implemented the International Numbering System (INS), which designates E-numbers to all approved food additives, establishing a uniform identifying system utilized by numerous countries for streamlined recognition and classification. In the USA, the Food and Drug Administration (FDA) makes sure that food additives are safe by establishing exact limits on the permissible quantity of each additive in food products and issuing rules for labeling items that contain them (Vinay et al., 2025).

Bread and fermented foods have been an important part of diets since ancient times. At first, they were valued for their energy content and then acknowledged for their nutritional advantages. This change underscores their crucial role in promoting general health. Currently, people want healthier foods that are low in calories and high in fiber and antioxidants. Food makers employ additives such as oxidants, enzymes, emulsifiers, and hydrocolloids to improve quality and extend shelf life (Vinay et al., 2025).

1.3.1 Hydrocolloids

Hydrocolloids are a safe and effective way to thicken, stabilize, and gel wheat bread. They stabilize emulsions and gelatinize starch by interacting with gluten proteins; this interaction might alter the rheology of the dough. Hydrocolloids improve bread volume, moisture retention, texture, and anti-staling (Conte et al., 2019). Based on the findings of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), hydrocolloids are classified as low-toxic and non-genotoxic. Cumulative exposure is safe for the general population, and chronic toxicity testing indicates that high

consumption levels do not have any adverse effects, and there is no evidence of reproductive/developmental effects or carcinogenic effects. A numerical Acceptable Daily Intake (ADI) is not necessary because there are only minor safety concerns at normal consumption levels (Ateş and Oner, 2018; Younes et al., 2018).

1.3.1.1 Carboxymethyl Cellulose (E466)

Carboxymethyl Cellulose (CMC) is a chemically modified cellulose derivative that adds carboxymethyl groups onto the cellulose backbone that enhances water solubility and thickening. It helps the dough absorb more water, making it softer and more pliable. It also makes the bread's crumb structure denser (Mohammadi et al., 2014). CMC affects how long dough takes to form (Ozkoc and Seyhun, 2015), makes mixing easier, and increases shelf life through moisture preservation and barrier coatings. While there is no defined ADI, the safety risks remain minimal at the observed consumption levels of 12–15 g/day (Vinay et al., 2025).

1.3.1.2 Hydroxypropylmethylcellulose (E464)

Hydroxypropylmethylcellulose (HPMC) is a non-ionic modified cellulose with methyl and hydroxypropyl groups that improves surface activity and hydration responsiveness (Morreale et al., 2018). It establishes hydrophilic linkages with diverse flours, which improves the water absorption of gluten-free breads (Abdollahzadeh et al., 2024). HPMC acts as a gel-like matrix, strengthening the dough's structure, retaining the gas that is created during fermentation, and enhancing its texture. It decreases stickiness and enhances the formation of shapes such as loaves or rolls, with standard concentrations ranging from 0.5 to 2% relative to flour weight. In addition to its advantages in water retention and dough flexibility, HPMC also acts as an anti-staling agent, which delays the hardening of crumb (Gómez and Román, 2018). Modified celluloses such as CMC and HPMC slow down the hardening process (Ferrero, 2017), make cells more stable, and have almost no safety concerns at the recommended dosages of 12–15 g/day.

1.3.1.3 Xanthan Gum (E415)

Xanthan Gum (XG) is a polysaccharide synthesized via the fermentation of simple sugars by Xanthomonas campestris. It appears as a loose powder that ranges in color from white to cream; it dissolves in water but is insoluble in the majority of organic solvents. XG has important shear-thinning properties and high low-shear viscosity, which make suspensions and colloidal solutions more stable (Kohajdová and Karovičová, 2009). Adding it to dough makes it better by increasing its ability to absorb water, hold gas, and stay stable against enzymes, shear, and freeze-thaw cycles. The standard application ranges from 0.5% to 1.5% of the total flour weight (Kaur et al., 2015), with elevated concentrations advised for gluten-free formulations. Despite It doesn't hold water as well as some cellulose derivatives, but it works better than locust bean and k-carrageenan (Maleki and Milani, 2013; Turabi et al., 2010). XG works well with other hydrocolloids in gluten-free

bread to improve viscosity and crumb tenderness (Gómez et al., 2007; Mancebo, 2015). ADI value for XG has not been established.

1.3.1.4 Carrageenans (E407)

Carrageenans (CA) are linear sulfated polysaccharides obtained from red seaweeds, comprising 18–40% sulfate and consisting of galactose and 3,6-anhydro-galactose units linked by alternating glycosidic bonds (Khalil et al., 2018; Wüstenberg, 2014**).** Their derivatives include κ-CA, iota-CA, and λ-CA. Each of them has gel properties that depend on the sulfate content. For example, κ-CA produces rigid gels, ι-CA makes flexible gels, and λ-CA results in viscous solutions (Necas and Bartosikova, 2013). κ-CA, typically utilized at 0.5% to 3% of flour weight, improves texture and moisture retention but exerts minimal influence on the characteristics of gluten-free bread. Its stability during freezing and thawing is better than that of other hydrocolloids, which is important for keeping frozen baked goods fresh (Salehi, 2019; Wüstenberg, 2014**).** The ADI for carrageenan is 75 mg per kilogram of body weight.

1.3.1.5 Guar Gum (E412)

Guar Gum (GG) is a polysaccharide made from the seed endosperm of Cyamopsis tetragonolobus. It has D-mannose and D-galactose in a 1.6:1 ratio. This polymer possesses a β-(1-4) linked linear backbone (Thombare et al., 2016), yielding high viscosity at low concentrations; these characteristics make it a good thickening, stabilizing, and water-binding agent. It is insoluble in organic solvents or hydrocarbons but rapidly hydrates in water to produce high-viscosity colloidal solutions without forming a gel. In bread production, GG improves mixing tolerance, moisture retention, and shelf life (Maity et al., 2018), and it is often utilized at 0.3% to 1% of the flour weight. Temperature, pH, and specific ions alter its effectiveness, and no recommended ADI exists for it.

1.3.1.6 Locust Bean Gum (E410)

Locust Bean Gum (LBG) is a natural polymer that comes from carob tree seeds, mostly consisting of D-galactose and D-mannose in a ratio ranging from 73:27 to 86:14, which gives it thickening and stabilizing properties. It is water-soluble in a wide variety of pH levels (Chawla and Patil, 2010), but it needs to be heated to more than 80°C for total disintegration (Wüstenberg, 2014). LBG collaborates efficiently with κ-CA and XG to make elastic gels exhibiting minimal syneresis. Its incorporation into frozen dough improves flexibility, increases the volume of the bread, and reduces proofing duration (Salehi, 2019). No ADI has been established.

1.3.2 Emulsifier

Emulsifiers are important substances in bread production, lowering surface tension at hydrophobic-hydrophilic interfaces. They promote the development and stability of air bubbles in dough, essential for adequate expansion during fermentation and baking. This makes the crumb softer, which helps it stay fresh longer, resist staling, and enhance moisture retention. Emulsifiers improve dough manipulation and consistency while promoting a finer crumb texture and increased freshness. Some common emulsifiers are Diacetyl Tartaric Acid Esters of Monoglycerides (DATEM), polysorbates, Sodium Stearoyl Lactylate (SSL), Mono- and Diglycerides (MDG), lecithin, and sucrose esters (Vinay et al., 2025).

1.3.2.1 Lecithin (E322)

Lecithin, a natural phospholipid made from maize or soybean oil, was the first emulsifier used in the baking industry. The emulsifying properties arise from the interactions between its phosphate and fatty acid groups. Lecithin improves darker and mixed-grain breads by enhancing water hydration, prolonging shelf life, emulsifying lipids, stabilizing gluten, and increasing loaf volume, with peak efficacy at a 0.2% incorporation in flour. It has a natural quality that appeals to people who like clean labeling, but it doesn't contribute much to strengthening the dough (Hui et al., 2008). Excessive amounts might negatively affect loaf volume and crumb quality, while gluten-free loaves can include up to 9% of it (Román et al., 2019). Despite having little effect on dough strength, its all-natural composition appeals to customers who prefer clear labels. Since there is no evidence of genotoxicity or cytotoxicity at higher dosages in animal studies, the European Food Safety Authority (EFSA) has not determined an ADI for lecithin (Vinay et al., 2025).

1.3.2.2 Diacetyl Tartaric Acid Esters of Monoglycerides (E472e)

Diacetyl Tartaric Acid Esters of Monoglycerides (DATEM) is a glycerol ester generated from mono- and diacetyl tartaric acid and consumable fatty acids and is commonly used in yeast-leavened foods like white bread. The recommended dose ranges from 0.25% to 0.50% relative to the weight of the flour. DATEM is available in a variety of forms, such as viscous liquids and powders, and exhibits superior hydrophilicity compared to MDG (Gioia et al., 2017). Adding 0.4% DATEM can increase the volume of a loaf by around 10%, improving dough stability and strength (Tebben, 2022); however, it may result in a coarser crumb texture and the formation of holes when utilized with inferior flours. DATEM is especially useful for producing crispy breads and enhancing a more open crumb structure in white loaves (Hui et al., 2008).

1.3.2.3 Stearoyl Lactylate

Stearoyl Lactylate (SL) is an anionic emulsifier synthesized from stearic acid and lactic acid, with the use of sodium or calcium as catalysts. It has 3.5 to 5% sodium or calcium, 34% lactic acid, and

an ester value ranging from 150 to 190. At a neutral pH, SSL dissolves in water more easily than Calcium Stearoyl Lactylate (CSL), whereas CSL dissolves in oil more easily (Arshad et al., 2019), SL enhances dough viscoelasticity by forming hydrophobic bonds with wheat proteins and inhibiting retrogradation through the formation of an insoluble complex with starch. This enhances dough strength, softens the crumb structure, prolongs mixing duration, and reduces proofing periods (Arshad et al., 2019). Using SL increases loaf capacity by creating smaller air cells and improves dough handling, which in turn reduces crumb hardness and staling, with a maximum recommended concentration of 3 g/kg or 3 g/L in bread (EFSA Panel on Food Additives and Nutrient Sources added to Food [ANS], 2013).

1.3.2.4 Mono- and Diglycerides (MDG) (E471) (Distilled Monoglycerides (DMG) and Ethoxylated Monoglycerides (EMG)

About 70% of worldwide food emulsifiers come from MDG, with 60% of monoglycerides utilized in bakeries—20% for cakes and 40% for bread. These emulsifiers are produced via the esterification of triglycerides with glycerol, resulting in a combination of mono-, di-, and triglycerides that stabilize oil/water emulsions (Gioia et al., 2017). Glyceryl monostearate enhances bread's softness and elasticity with a negligible effect on loaf volume. The effectiveness is due to the delay of starch retrogradation, with the best dose being 0.2% of the base flour (Hui et al., 2008).

1.3.2.5 Polysorbate

Polysorbates (PS) are non-ionic surfactants consisting of partial esters of fatty acids and sorbitol. The food industry makes heavy use of PS derivatives as emulsifiers, particularly PS 20, PS 60, and PS 80, to enhance dough properties, including loaf softness and volume enhancement during baking and fermentation (Tebben et al., 2018). They diminish dough development duration, adhesiveness, and thickening of cell walls without compromising the crumb texture during storage. The recommended usage ranges from 0.5 to 1% by weight of flour, with a daily consumption of 25 mg/kg for polysorbate being considered acceptable by JECFA. Excessive doses may result in gastrointestinal and metabolic complications (Vinay et al., 2025).

1.3.3 Enzymes

Recently, the demand for natural ingredients has led to an augment in the use of enzymes in wheat flour. Enzymes, predominantly found in the germ and aleurone layers, function as substitutes for chemical agents such as hydrocolloids and emulsifiers. Proteases, oxidoreductases, and amylases are the main types of enzymes that improve loaf volume and dough properties (Gioia et al., 2017). Critical determinants affecting enzyme activity include temperature, pH, water activity, and enzyme concentration.

1.3.3.1 Amylases

The staling of bread, which is mostly produced by amylopectin retrogradation, shortens bread's shelf life and causes economic losses for the industry. To address this, α-amylases are used to break down starch into low molecular weight dextrin and maltose. These enzymes, sourced from fungi, cereals, or bacteria (Miguel et al., 2013), hydrolyze α-1,4-glycosidic linkages, thereby increasing loaf volume and reducing staling (Tebben et al., 2018). β-amylase further transforms dextrin into maltose; nevertheless, its partial hydrolysis constrains efficiency. Fungal glucoamylases target α-1,6 links, but bacterial amylases from Bacillus subtilis have superior stability and efficacy in starch degradation (Chandrasekaran, 2020). Although bacterial amylases significantly enhance shelf life, using too much of them may result in unfavorable dough texture (Hui et al., 2008). According to some regulations, the EU classifies these enzymes as processing aids.

1.3.3.2 Protease

Protease enzymes are essential in the baking industry, as they improve numerous qualities of dough. They enhance many properties, such as dough consistency, diminish mixing duration, augment uniformity, and elevate the flavor and texture of bread by hydrolyzing peptide bonds and lowering gluten elasticity, hence minimizing dough shrinkage (Miguel et al., 2013). Malt proteases are used in weak flours to enhance dough softness. Although fungal proteases derived from Aspergillus oryzae are heat-sensitive, which limits their effectiveness to the baking and dough stage, targeted applications of protease from Aspergillus oryzae at low temperature (25-45°C) can enhance dough expansion and gas retention, leading to a larger loaf of bread. In the USA, bacterial proteases produced by Bacillus subtilis are commonly used to reduce rusk dough and prevent laminated dough flaws. Additionally, the color and flavor of the bread are enhanced by the Maillard process, which uses amino acids that are produced during proteolysis (Gioia et al., 2017; Aoki et al., 2020). Moreover, the Thermoase treatment of Bacillus stearothermophilus improves dough characteristics and diminishes staling rates during storage, resulting in soft-textured goods (Kawamura-Konishi et al., 2013).

1.3.3.3 Lipases

Lipases, also known as triacylglycerol acyl hydrolases, catalyze the hydrolysis of triacylglycerol, yielding monoacylglycerol, diacylglycerol, glycerol, and free fatty acids. Lipases are found in cereal grains, but only in small amounts. Their utilization in food technology is comparatively novel in relation to proteases and α-amylases (Giannone et al., 2016; Melis et al., 2019; Moayedallaie et al., 2010). Lipases enhance dough stability and the gluten network, hence improving dough handling, machinability, loaf volume, density, and crumb quality (Dahiya et al., 2020). Since its inception in the 1990s, third-generation lipases have been designed to work better in modern dough processing while generating fewer short-chain free fatty acids, hence reducing

off-flavors during storage (Dura and Rosell, 2017). Lipases may function as antistaling agents, partially substituting emulsifiers in baked products (Bock, 2015).

1.3.3.4 Glucose Oxidase

Glucose Oxidase (GOX) is an oxidoreductase enzyme mostly derived from Aspergillus spp., facilitating the conversion of β-D-glucose into gluconic-D-lactone in the presence of oxygen. This process results in the production of gluconic acid and hydrogen peroxide, which facilitates the oxidation of gluten sulfhydryl groups (thiol group), leading to the creation of disulfide bonds and a drying effect in bread dough (Bock, 2015). GOX makes dough less stretchy and more resistant and improves bread volume by oxidizing water-soluble SH and promoting oxidative gelation of pentosanes (El-Rashidy et al., 2015). Its incorporation enhances water absorption, dough elasticity, and tenacity without influencing gluten protein extractability.

1.3.3.5 Hemicellulase

Hemicellulases are hydrolytic enzymes that catalyze the degradation of hemicellulose components, such as arabinoxylan and xylan, with xylanase, which is an important enzyme in making bread (Dhiman and Mukherjee, 2018). Xylanase transforms water-insoluble hemicellulose into a soluble state, improving bread volume and crumb consistency and decreasing dough stiffness and adhesiveness (Butt et al., 2008). Xylanase works best when combined with other enzymes; it has little effect when used alone. Combinations of α-amylase and glucose oxidase enhance dough flexibility and bread volume, whereas the incorporation of α-amylase and lipase in the straight dough method improves bread shelf life and creates a stable amylose-lipid complex (Vinay et al., 2025).

1.3.4 Oxidizing and Reducing Agent

Oxidizing agents improve the gluten network by forming disulfide linkages through oxidizing thiol groups. The mixing time is reduced, and the natural aging of flour is accelerated as a result. They further bleach carotene and xanthophyll colors in flour (Joye et al., 2009). Common oxidants such as Potassium Bromate (PB) and ascorbic acid enhance dough fermentation, elasticity, and stability, leading to improved strength, elevated oven rise, and superior crumb texture. The application of these chemicals depends on the quality of the flour, the method of preparation, and regional restrictions (Gioia et al., 2017).

1.3.4.1 Oxygen

The integration of molecular oxygen during dough mixing is essential in bread production, since it strengthens the dough by gluten cross-linking. Combining in an oxygen-saturated environment leads to reduced extensibility and increased resistance. On the other hand, starch granules that

absorb oxygen improve loaf volume by promoting the cleavage of disulfide bond. Bread quality can be negatively impacted by early oxygen desorption, which can happen as a result of either insufficient or excessive mixing. Molecular oxygen functions as the principal oxidant employed in the baking industry, essential for many biological and chemical processes (Vinay et al., 2025).

1.3.4.2 Ascorbic Acid (E300)

Ascorbic acid, or vitamin C, is the most extensively employed enhancer in the baking sector, especially in countries where its use is regulated. It is usually used at concentrations between 20 and 150 ppm, depending on the type of flour and how it is stored. It is not as effective as bromate at making loaves bigger. Most of the industrial ascorbic acid is produced from glucose by fermentation and chemical processes. It functions as an oxidizing agent, enhancing dough strength and improving crumb structure and color while decreasing stickiness (Sahi, 2014). Its effectiveness is linked to interactions within the gluten network, facilitating the formation of a more elastic dough and improving volume and texture, especially in the presence of oxygen (Beghin et al., 2022; Dagdelen and Gocmen, 2007). Ascorbic acid is acknowledged by the FDA as Generally Recognized as Safe (GRAS).

1.3.4.3 Azodicarbonamide (E927)

Azodicarbonamide (ADA) functions as a bleaching agent and dough conditioner in bread production, improving dough strength by oxidizing thiol groups in flour proteins. It enhances the processing properties and gas retention of lower-quality flours by altering the dough's rheology, which raises its storage and loss moduli (Joiner et al., 1963). Using ADA wisely can improve the volume and crumb structure of bread (Beghin et al., 2022); however, excessive quantities may diminish volume. Although efficient in the Chorleywood bread process, its rapid reaction rate may hinder alternative procedures. It is important to note that ADA is prohibited in EU countries due to health hazards associated with semicarbazide, a byproduct of its reaction. However, it continues to be utilized in other regions (Sahi, 2014).

1.3.4.4 L-Cysteine (E920)

L-cysteine is an amino acid that occurs naturally and is used as a reducing agent in bread-making, improving dough extensibility and diminishing elasticity. It promotes disulfide bond catalysis among gluten-forming proteins, resulting in a more pliable dough. Overuse can make dough sticky and difficult to handle, especially when working with dense flours that have low gluten extensibility, causing diminished gas retention and volume. L-cysteine makes dough stickier and easier to work with, although it might make it less elastic and less tolerant of mixing. Although mixing degrades high molecular weight glutenins to enhance flexibility, it can decrease bread volume and make the crumb grittier if used alone. To achieve the best results, it should be used with oleo gels or thickening agents. The FDA recognizes L-cysteine as GRAS for baking uses,

with an appropriate daily consumption of 2.2 grams (Vinay et al., 2025). There are other types of oxidizing and reducing agents, such as PB, chlorine dioxide, and Potassium Iodate (PI), which we will discuss later in the next section.

1.3.5 Leavening Agents

A leavening ingredient enhances the volume of dough and batter by generating froth; hence, it softens and lightens the combinations. It serves as a nucleation seed for gas production or enhances shape and texture by gas expansion via chemical reactions (Gujral and Singh, 1999). You can also add air through mechanical action instead of using a leavening agent.

1.3.5.1 Yeast

The utilization of yeast in bread production, originating 6000 years ago with the ancient Egyptians (Cauvain, 2007), predominantly entails the strain Saccharomyces cerevisiae, employed at approximately 1% of the flour weight. The best temperature for yeast activity is between 34 and 38°C, and the best pH level is between 4.5 and 5.5. During fermentation, yeast generates zymase to decompose carbohydrates into ethanol and carbon dioxide (CO_2), enhancing crumb structure and increasing bread volume (Neeharika et al., 2020). Excess sugar and sodium chloride can harm yeast activity by generating elevated osmotic pressure. Baker's yeast comes in several forms with different amounts of moisture. Compressed yeast (67–71% moisture) is the most effective leavening agent, but it doesn't work well at high temperatures (Mietton et al., 2022). Active dry yeast (4–8% moisture) lasts longer. In sourdough manufacturing, yeast and lactic acid bacteria collaborate, with yeast producing CO_2 and bacteria imparting a sour flavor. Traditional breadmaking rarely makes use of alternative leavening agents.

1.3.6 Preservatives

Preservatives are vital in bread production, as they prolong shelf life and preserve quality by preventing microbiological proliferation. Because it has a high-water activity of about 0.96, bread is susceptible to microbial contamination, resulting in deterioration and mycotoxin synthesis. Bread formulations often incorporate preservatives such as propionate, sorbate, acetate, and ascorbic acid to modify pH, inhibit microbial activity, and maintain the bread's safety and aesthetic appeal for eating (Vinay et al., 2025).

1.3.6.1 Sorbates

Sorbic acids and their potassium salts are recognized as safe and effective in inhibiting mold growth in baked goods, with sorbates exhibiting double the efficacy of propionates in specific applications. Nonetheless, their application may impact yeast, resulting in sticky dough and inconsistent baked goods. Suggested application concentrations vary from 0.001% to 0.3%.

Options to solve problems comprise the use of sorbate post-baking, combining with fatty acids, or utilizing encapsulated forms. Sorbate palmitate is known for its capacity to keep mold from growing without stopping fermentation. This is because it hydrolyzes during baking to create sorbic acid, which inhibits mold growth during storage (Vinay et al., 2025).

1.3.6.2 Propionates

Propionic acid and its salts (sodium, potassium, and calcium) are white powders with a cheese-like taste utilized as preservatives in bread. They help in preventing mold proliferation and bacterial degradation caused by Bacillus species, particularly B. subtilis and B. licheniformis. Calcium propionate can have a bad effect on the volume of a loaf by interfering with yeast activity and dough characteristics. Additionally, excessive use of propionic acid may induce propionic acidemia in children, resulting in complications such as learning disabilities, seizures, arrhythmias, gastrointestinal disturbances, and recurrent infections (Vinay et al., 2025).

1.3.6.3 Acetates

Acetic acid, present as a colorless liquid or crystalline solid, effectively combats bacterial spoilage in baking, especially the "rope" phenomenon, at a suggested concentration of 0.1–0.2% based on flour weight. Although it prolongs shelf life, excessive amounts may produce an unpleasant vinegar odor. Acetates, which are usually white crystalline granules, come in sodium, potassium, and calcium forms. They act as natural preservatives in sourdough by generating ethyl and isoamyl acetates. These characteristics offer both aesthetic allure and prolonged mold-free shelf life for bread (Vinay et al., 2025).

3.7 Advanced Food Additives in Bread-Making

1.3.7.1 Bacterial Nanocellulose

Bacterial Nanocellulose (BNC) is a biopolymer synthesized by aerobic bacteria, primarily Gluconacetobacter spp, using D-glucose as a substrate (Klemm et al., 2006). The FDA has classified it as GRAS. It is composed of a three-dimensional network of nanofibers that are 2 to 4 nm thick and fewer than 100 nm wide (Shatkin, 2020). BNC functions as a thickening, gelling, stabilizing, and suspending ingredient in product formulation. It functions as a fat substitute and improves water retention. In bread-making, the incorporation of BNC at 0.14% increases specific volume and moisture content (Corral et al., 2017), yielding a softer and more porous texture attributed to improved gas retention and a more robust dough cross-linking network.

1.3.7.2 Cistus Incanus

Cistus Incanus L. (CI) is a medicinal shrub recognized for its antioxidant, antibacterial, anti-inflammatory, anti-diabetic, anti-ulcerogenic, and cytotoxic attributes (Jeszka-Skowron et al., 2018). It has many antioxidants, such as flavonoids, ellagic acid, and gallic acid. CI is employed in herbal infusions, food additives, and nutraceutical goods, such as dietary supplements and herbal teas. Adding 1–5% CI leaves or extract to dough might help bread rise by making it absorb more water (Viapiana et al., 2017). However, at higher concentrations, the loaf volume could be slightly reduced, and textural aspects like hardness and springiness could be affected (Caccak-Pietrzak et al., 2019). In summary, up to 3% CI can enhance the nutraceutical attributes of bread without adversely affecting its quality. Nonetheless, elevated amounts of CI may disrupt the absorption of proteins and minerals (Vinay et al., 2025).

1.3.7.3 Propolis

Propolis is a resinous compound synthesized by honeybees from botanical sources, utilized for hive maintenance and defense. The chemical composition depends on the plant species and the environmental factors. It includes polyphenols, terpenoids, sesquiterpenoids, cinnamic acid, essential oils, aromatic acids, benzaldehyde resins, sterols, minerals, and other organic substances (Wagh, 2013) that have antibacterial, antiangiogenic, cardioprotective, hepatoprotective, and antioxidant effects (Daleprane and Abdalla, 2013). In bread production, the use of propolis increases mineral content without modifying nutritional composition, influences texture, and elevates amylopectin crystal formation (Hosseini Khabbazi, 2023); hence, it enhances antioxidant capabilities (Föste et al., 2020). Propolis-enriched bread provides a natural preservation alternative to synthetic preservatives, attracting consumers desiring healthier choices.

1.3.7.4 Transglutaminase

The enzyme EC 2.2.13, synthesized by Streptomyces mobaraensis is a Transglutaminase (T Gase) that facilitates acyl transfer processes involving protein-bound glutamine. This method makes covalent cross-links between different amino compounds, which make high molecular weight polymers that improve food texture and rheological characteristics (Gharibzahedi et al., 2018). In the creation of gluten-free bread, T Gase optimizes moisture retention, facilitates less yeast utilization while preserving structure and texture, and improves attributes such as mixing stability, dough strength, and loaf volume (Dłużewska et al., 2015). The enzyme's efficacy varies depending on the protein source and is suitable for gluten-free and low-gluten-quality flours. T Gase, as a protein, is inactivated through baking and was granted GRAS designation by the FDA in 1998(Vinay et al., 2025).

1.3.7.5 Laccase

Laccase (LAC, EC 1.10.3.2) is a copper-dependent enzyme that oxidizes phenolic compounds through the production of reactive radicals. It affects substrates like tyrosine in proteins and arabinoxylan linked to ferulic acid esters, enhancing polymerization and enabling radical interactions (Mattinen et al., 2005). LAC treatment reduces extensibility and augments dough hardness, with firmness declining over time due to radical-induced depolymerization of the arabinoxylan network. Thermal inactivation of LAC post-gel formation stabilizes gel firmness; hence, it improves bread volume. These effects are linked to the crosslinking of ferulic acid residues in dough. They show a strong preference for arabinoxylan as a substrate over gluten, but they also change the quality of gluten (Vinay et al., 2025).

1.4 Flour Additives Studied in this Research

1.4.1 Potassium Bromate

Potassium bromate (PB), often known as bromate in the industry, has been widely employed in baking as an oxidizing agent. It manifests as an odorless, white crystalline or powdered substance, with excellent solubility in water and reduced solubility in acetone, ethanol, methanol, dimethyl sulfoxide, and toluene (Sahi, 2014; Vinay et al., 2025). For more than ninety years, PB has been extensively utilized as a flour additive due to its efficacy as a dough improver and maturation agent (Nkwatoh et al., 2023; Oloyede and Sunmonu, 2009). It diminishes the aging duration and is extensively utilized for fermentation and proofing dough in the baking process (Vinay et al., 2025). During the late dough stage, PB oxidizes the thiol groups of gluten proteins in flour to disulfide linkages, leading to an increase in the strength of the protein network and resulting in decreased extensibility and increased elasticity and dough strength, so enabling the dough to retain the carbon dioxide gas generated by the yeast during leavening (Abu-Obaid et al ., 2016; Alli et al., 2013; Chauhan and Jain, 2016; Ojo et al., 2013), boosting gas cell structure, and producing bread with optimal loaf volume and a consistent fine-crumb structure (Vinay et al., 2025). Bromate is acknowledged as a slow-acting oxidant and cheap relative to others but effective, exhibiting negligible impact during the mixing period. It is engaged during the latter phases of proofing and baking. The principal effect is evident during extended proofing periods and the initial phases of baking. Bromate activity is contingent upon temperature; as temperature rises, activity correspondingly increases. Potassium bromate decomposes with distilled water at 350°C to produce oxygen and potassium bromide. It deteriorates in wheat flour around 150-200°C due to the catalytic activity of metal ions present, including copper, iron, magnesium, aluminum, and zinc. Combining bromate with ascorbic acid can enhance its efficacy and activity (Sahi, 2014; Vinay et al., 2025). That’s why PB is likely the most economical and efficient oxidant utilized in bread manufacturing, and bakeries capitalize on these advantageous characteristics for financial gain (Nkwatoh et al., 2023).

Despite its multiple benefits, excessive PB usage leads to residual levels in bread that may have detrimental effects. The International Agency for Research on Cancer (IARC) has classified PB as potentially carcinogenic to humans. (Class 2B) (Ben Saad et al., 2015; Sahu et al., 2016). Therefore, numerous organizations, agencies, and governments have prohibited its usage as a flour ingredient, such as the EU, along with the JECFA, Food and Agriculture Administration (FAO), and World Health Organization (WHO) panel. On the other hand, the concentration of bromate is contingent upon national restrictions in other countries (Vinay et al., 2025). Thus, certain nations have instituted maximum permissible levels for completely baked goods; for example, the maximum allowable values of PB in baked goods are 10 mg/kg in Japan (Kurokawa et al., 1990; Oyekunle et al., 2014), and the FDA has established the permissible limit at a maximum of 50 mg/kg of flour or 75 mg/kg (Vinay et al., 2025). Notwithstanding the prohibition or limitations on PB as a flour ingredient, bakers continue to utilize substantial amounts in bread production (Elsheikh et al., 2016).

The investigations underscore the necessity of assessing the human health concerns associated with PB for bread consumers, considering that bread is a fundamental food item worldwide and because PB is a prevalent flour addition utilized by bakers in several places. Furthermore, several nations, such as Cameroon, have neither formally prohibited PB as a flour additive nor defined allowable concentrations in baked goods (Nkwatoh et al., 2023). In which numerous studies indicate that PB has both acute and chronic health effects. Acute signs of PB intoxication encompass abdominal pain, diarrhea, irritation of the upper aerodigestive mucous membranes (inflammation of the upper digestive tract), vomiting, nausea, deafness, anuria, oligonuria, hypotension, and vertigo; thrombocytopenia; depression of the central nervous system; and other related health problems (Sahu et al., 2016). Free radicals that are produce from bromate cause nephrotoxicity and cancer (Campbell, 2006; Chipman et al., 2006; Moubaraka et al., 2020). It also causes kidney cell tumors, mesotheliomas, and thyroid follicular cell tumors in rats (Achukwu et al., 2009). It degrades vitamins A2, B1, B2, E, and niacin, which are the main vitamins available in bread (Kujawska et al., 2013). Several studies have reported significant differences in the essential fatty acid content of flour treated with bromate or in bread made from that flour (El-Deeb and Abd-El-Hafez, 2015). It is worth mentioning that heat during baking catalyzes the transformation of PB into the less hazardous compound potassium bromide (Ati-Hellal, 2018), deemed safe for ingestion. The total conversion of bromate to bromide cannot be presumed, as it is contingent upon the baking temperature, duration, form (powder or liquid), and quantity of bromate utilized. Also, the degradation of bromate can be accomplished using certain reducing agents and ferrous sulfate.

1.4.2 Alloxan

Alloxan (2,4,5,6-tetraoxypyrimidine or 2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine synthesized through the oxidation of uric acid, potentially existing in a hydrated form. Since 1943, research has indicated that alloxan is toxic to pancreatic β-cells, which leads to diabetes; this is

why it is used to cause Type-I Diabetes Mellitus in animals (Carvalho et al., 2003; Dunn et al., 1943; Giaccone et al., 2017; Mir et al., 2013; Szkudelski, 2001). Alloxan resembles glucose and exerts two distinct effects on diabetes pathology: firstly, it selectively inhibits glucose-induced insulin secretion through the specific inactivation of glucokinase (Dhanabal et al., 2007; Giaccone et al., 2017; Zhou et al., 2017), and secondly, it enhances the formation of Reactive Oxygen Species (ROS), which can oxidize nucleic acids, proteins, and lipids, thereby damaging numerous cellular components (Kim et al., 1994; Giaccone et al., 2017). Moreover, numerous studies have demonstrated that alloxan possesses carcinogenic properties in rats and fish; additionally, it can produce adenohypophysis cancer in mice (Giaccone et al., 2017). Furthermore, a study discovered elevated concentrations of alloxan in the bloodstream of children with insulin-dependent diabetes mellitus, correlating with the start of the condition (Giaccone et al., 2017). It is important to note that alloxan is a small byproduct of protein oxidation; hence, it may be created during the bleaching procedures of alimentary flour for color and dough enhancement, potentially resulting in a toxicant (Banu and Sasikala, 2012). As we know, flour is made from freshly ground wheat that contains carotenoids, which is why it appears pale yellow. This flour makes sticky dough that is difficult to cook. When flour is stored, it goes through a sequence of oxidative processes, including thiol groups of proteins and carotenoids. This process makes the flour naturally age, making it white, bulky, and soft, which is better for making baked goods (Giaccone et al., 2017). To accelerate this process, chlorine dioxide- which is an additive- is significantly used for enhancing dough characteristics, mostly by bleaching carotenoids in wheat, which influences the flour's color and reduces its yellowness, with a maximum permissible content of 2,500 mg/kg (Vinay et al., 2025).

The Food and Drug Administration includes chlorine on its list of food additives (Fukayama et al., 1986). That's why chlorine and hypochlorites are consider safe to use in food processing in the USA. However, many governments have prohibited chlorine dioxide and chlorine gases due to their potential harmful effects (Giaccone et al., 2017). Gliadin proteins are more sensitive to chlorine than glutenins; whereas chlorine oxidizes aromatic amino acids and thiol groups, disulfide bonds remain unaffected. Additionally, these hazardous chemicals impair the conjugated double bonds and oxidize the thiol groups of the gluten proteins. Furthermore, the chlorine processing includes breaking peptide bonds and hydrogen bonds, as well as examining aromatic amino acids. Oxidation reactions can alter flour components and produce harmful compounds such as alloxan (Fukayama et al., 1986; Giaccone et al., 2017). It is important to emphasize that chlorination enhances the aesthetic quality of bread, producing a white crumb, fine gas cells, increased loaf volume, and improved sensory characteristics. (Vinay et al., 2025).

1.4.3 Potassium Iodate

Potassium Iodate (PI), generally known as iodate, is a more potent oxidizing agent than bromates. During the baking process, iodate oxidizes free thiol groups of cysteine in the wheat protein chain and it reduces to iodide. The application of elevated iodate concentrations induces the polymerization of high molecular weight glutenin, hence inhibiting crosslink formation by

oxidizing thiol groups to elevated sulfur oxidation levels. Consequently, it impedes crosslinking, leading to reduced loaf volume in bread. The extractability of glutenin and gliadin diminished in iodate-treated flour throughout the mixing, fermentation, and baking processes. Despite being widely regarded as safe, it was suggested that for iodate the optimum level of addition is in the range of 10-20 mg per kg (Sahu and Saxena, 2016; Vinay et al., 2025). Potassium iodate is regarded as an excellent supplier of iodine. In 1965, the JECFA advised against the use of PI as a flour treatment agent due to the potential for increased iodine consumption. The committee deemed the application of a food additive, like iodine, to a staple material such as flour to be very undesirable due to its physiological relevance and potency. Its utilization may lead to a daily iodine consumption that is five to tenfold greater than the typically advised dose of 100-200 μg. Therefore, PI is not advised for use as a flour treatment agent in certain regions, including the United Kingdom (UK), the EU, Australia, and New Zealand (Sahu and Saxena, 2016). It is worth noting that there is no agreement on the maximum limit for using PI, but overall, some studies indicate it should be at most 45 mg/kg and other studies indicate that it is at most 75 mg/kg.

1.5 Applications of Infrared Spectroscopy in Quantitative and Qualitative Analysis

Infrared (IR) spectroscopy is a highly informative and non-destructive analytical method applicable across various scientific disciplines, including physics, chemistry, biology, and the environmental, pharmaceutical, material, and food sciences. The absorption of IR light by the sample, observed through molecular vibrations and transitions, facilitates both quantitative and qualitative analysis of various chemicals. Infrared spectra can be acquired from almost any kind of substance and in any physical state (solid, liquid, and gas), rendering this analytical technique highly versatile and powerful for the examination of inorganic and organic molecules, biological tissues, and metabolites within biological systems. The spectral range from 400 to 4000 cm^{-1} is referred to as the mid-IR region, while the interval from around 400 to 300 cm^{-1} is known as the far-IR. In the mid-IR spectrum, regions can be categorized based on specific criteria pertinent to the analysis of inorganic, small organic, and extraneous species: (a) the Functional Group (F/G) region, (b) the condensed phase inorganic region, (c) the near-IR region, and (d) the Fingerprint (FP) region. In qualitative evaluations, the F/G region is almost the most important because the established regions are different for each inorganic element (Shriner et al., 2003). Infrared absorptions typically manifest at low wavenumbers, absent prior corroborative spectral evidence (regions are generally narrower or enhanced with more pronounced peaks). Measurement and data analysis can be executed with minimal error, even utilizing small fiber or single reflection. Attenuated Total Reflectance (ATR) measuring cells and detectors with restricted wavelength ranges. To comprehend the interaction between infrared radiation and matter, it is essential to investigate the mechanisms underlying the absorption process, particularly those related to bands associated with (F/G). This understanding is vital for the development of qualitative methods and the selection of appropriate mathematical analyses for these bands (Johnson et al. 2023).

1.5.1 Basic Principles and Instrumentation

Spectroscopy primarily examines the interaction between electromagnetic radiation and matter, leading to the absorption of radiation energy by the latter. Infrared spectroscopy, also known as vibrational spectroscopy, is based on the idea that molecules absorb IR light, which causes the molecules' energy levels to vibrate. The transition frequencies are influenced by the types of atoms, their connectivity, and the molecular environment, resulting in distinctive spectral characteristics in the IR spectrum. The IR spectroscopy technique is extensively employed in both quantitative and qualitative analyses across diverse analytical domains (Shriner et al., 2003). Infrared spectroscopy can be categorized into many forms according to the method of interference pattern measurement. The two predominant instruments are dispersive IR spectrophotometers, which quantify the complete emission of the IR light source and isolate certain bands through a wavelength filter. Diode arrays or Fourier-Transform Infrared (FTIR) spectrophotometers delineate the spectral region of interest by employing monochromatic light for wavelength scanning to obtain a complete IR spectrum. A principle of diode arrays (or detector arrays for FTIR) is adequately robust to analyze drug substances. However, more complex designs are needed to measure dosage forms. The fundamental FTIR apparatus comprises a Michelson interferometer featuring a movable mirror, an IR light source, a stationary mirror, a sample-holding chamber, a beamsplitter, and an IR detector, along with accompanying electronics to convert the detector into a standard, visible output. The absorbance spectrum of the sample is generated by processing the interferogram data with software. A qualification tool and software are essential for the creation of dependable techniques and the validation of instruments, ensuring that the instrument hardware is undamaged and properly connected. There are standard test parameters for an FTIR qualification test. The predominant method for presenting samples in IR analysis is the potassium bromide (KBr) pellet, which is made from a homogeneous mixture of the solid sample and KBr that is compressed under high pressure; alternatively, the sample may be diluted with a reflecting agent to create a matrix. To make the IR instrument work properly, you need to clean and potentially dry inactive, powdered KBr for use in the pellets. These tasks can be done by using a vacuum desiccator and filtering through glass wool (Fahelelbom et al., 2022).

1.5.2 Quantitative Analysis Using IR Spectroscopy

The intended applications of IR spectroscopy have garnered considerable attention throughout its development over the decades. The structure, function, and reactivity of compounds are all linked to their molecular vibrations. You can learn more about these by looking at the 2D frequency map of the absorptive IR spectra. The atomic makeup, bond, and molecular form of the material have a big effect on the position, intensity, and polarity of the IR bands. The primary focus is the application of IR spectroscopy for quantitative analysis. Infrared spectroscopy can quantify the concentration of an analyte inside a mixture. The quantification of constituent concentrations is a crucial aspect of analytical chemistry. The primary aim of an analytical chemist is to ascertain the quantity of the analyte present. Currently, IR spectroscopy is employed for quantification across

various domains. Infrared-based quantification is an analytical approach that uses different calibration methods to provide a precise, linear, and accurate link between the concentration of the analyte and the IR measurement (Sun, 2009). Qualitative analysis entails identifying the constituents of a sample when there is prior knowledge of potential analytes but not their quantities. If the other analytes are already identified and the method is not new, this can be considered semi-quantitative or multicomponent analysis instead of conventional qualitative analysis. To execute quantitative analysis, an IR method must adhere to a systematic approach for method validation encompassing all procedures from sampling, sample preparation, and analysis to final reporting, particularly concerning the quantities of polyherbal and polypharmacy in a formulation. For intricate analyses, such as the in vitro chromatographic dissolution profile in a biowaiver test, a comprehensive qualitative analysis alternative to UV-visible spectroscopy could be infrared spectroscopy (Eserian and Lombardo, 2015).

1.5.2.1 Calibration Methods

Infrared spectroscopy provides a detailed and significantly overlapping quantitative study for structural differentiation. As labor costs continue to decline, the requirement for quantification appears to diminish, particularly for complex mixes. Conversely, the discipline of process analysis has a lot of potential for applications that do simultaneous, rapid, real-time, and quantitative screening. Calibration is typically employed to facilitate quantitative analysis by connecting the absorbance of a sample obtained by a specific experimental method with the analyte concentration in the mixture through a linear equation. In IR spectroscopy, reference spectra with concentration for individual quantitative analysis are available, and the generally employed calibration methods are as follows (Johnson et al., 2023).

Calibration curves: A linear equation representing absorbance as a function of analyte concentration. The range and standards are the two essential components involved in the development of an appropriate calibration curve. Linearity and the accuracy of the equipment are other critical factors. The predominant challenges in probing and qualitative analysis are the inadequate sample measurement, the matrix effect, and the limited resolution of the fitting spectrum. A recently emerging multivariate calibration methodology in the realm of IR spectroscopy improves the method's efficacy but is inherently more complex than the original technique. The commercially available software package enhances data collecting, management, and interpretation; however, a suitability study conducted in accordance with government guidelines or other suitable methodologies is recommended to validate the analytical and documentation procedures (Johnson et al., 2023).

1.5.3 Qualitative Analysis Using IR Spectroscopy

Unfortunately, the evaluation of the functional group signal typically does not provide direct information on the molecular structure. Conversely, the molecular structure of a compound can be

inferred from the correlation of several signals detected in the far-IR, mid-IR, and near-IR regions. Because the F/G signal doesn't give direct information about the molecular structure, it's also not able to do quantitative analysis in the associated spectrum range. Nevertheless, the quantity of each F/G present in the compound under investigation can be approximately quantified. The FTIR approach is highly effective for analyzing solids, liquids, and gases in the frequently utilized mid-IR range. The primary benefits of IR spectroscopy for qualitative and quantitative analysis are the minimal requirement for sample preparation, the rapidity of analysis, and the low background interference without the necessity of markers. Consequently, the technique has gained significance and has been utilized in the analysis of solid drugs and pharmaceuticals to produce a direct, accurate, exact, and stable quality product (Shriner et al., 2003). Infrared spectroscopy can be employed, with some changes, to ascertain the bioavailability and pharmacological release of a drug. Qualitative analysis will aid industries and environmentalists in the subsequent case studies: Pharmaceutical formulations can benefit from the assessment of bioavailability and drug release levels through qualitative analysis. Forensic applications encompass the analysis of restricted chemicals in blood, particularly when the separation of volatile medications is unattainable. The analysis involves the examination and measurement of petroleum-derived substances used in solar cells with ultra-thin sheets. The detection of process contaminants in bulk pharmaceuticals and medicinal products. Assessment of polymorphism in nanogram and sub-nanogram microvessels of pepper spray and liquid specimens. Due to its capacity to identify F/G and specify and qualify chemical components, isomers, and multiple formulations within a system, infrared analysis has been employed in the examination and identification of Unregulated Organic Chemicals (UOCs). The analysis in all previously examined cases relies on IR spectra library data, the identification of an unknown using the same or bracketing keys and is conducted in the absorption mode of energy or ET mode. The establishment and enhancement of a database of IR spectra, incorporating retention index, retention time, and quantitative and qualitative data, is strongly advocated for the qualitative analysis of excipients, drugs, and controlled substances. This approach would ensure high specificity and sensitivity, as most formulations approved for human and animal consumption have undergone testing via IR spectroscopy. The accessibility and dissemination of data for the spectral libraries and necessary software are critically important (Yang et al., 2022).

1.6 Validation of the Proposed Method

Validation is a practical method employed to ascertain that a technique is appropriate for use as a quality control instrument. The aim of any analytical measurement is to acquire consistent, reliable, and precise data. Validated analytical methodologies are crucial in attaining this objective. An analytical method comprises techniques, procedures, and protocols. Analytical method validation includes the assessment of accuracy, precision, Limit of Detection (LOD), Limit of Quantification (LOQ), linearity, and range. The outcomes of method validation can be employed to assess the reliability, quality, and consistency of analytical results, which is a fundamental aspect of sound analytical practice (Yang et al., 2022). Validation of analytical procedures is mandated by numerous regulations and quality standards affecting laboratories. The FDA defines validation

as a mechanism for production and process control aimed at ensuring that drug products possess their identity, quality, strength, and purity. The significance of validation lies in quality assurance, time-constrained optimization of processes, and minimization of quality expenses. Nominal discrepancies and bottlenecks reduced batch failures, enhanced efficiency and productivity, and decreased rejections. More production. Avoiding spending money on capital fewer complaints about mistakes in the process. Both the process and the resulting goods undergo less testing. Accelerated and dependable initiation of new equipment facilitated scale-up of formulation development efforts, facilitated equipment maintenance, and enhanced employee cognizance of procedures. Scientists should initiate validation in the lab, where they can observe the product's functionality and the necessary conditions for its operation. They learn when the product stops working, becomes unreliable, or starts to lose quality. After the laboratory has defined the boundary processing criteria, this information can be utilized to construct validation requirements. The validation of a system is an ongoing process. Once a new system and process demonstrate their effectiveness, they require continuous maintenance, regular calibrations, and adjustments. Consequently, the process is perpetually subject to examination and ongoing assessment (Chavan and Desai, 2022).

1.6.1 Key Parameters of the Analytical Method Validation

Understanding the parameters or features involved in the validation process is essential. The performance parameters are categorized as follows: accuracy, precision, specificity/selectivity, LOD, LOQ, linearity, range, robustness, repeatability, reproducibility, intermediate precision, ruggedness, and system suitability testing (Chavan and Desai, 2022).

1.6.1.1 Accuracy

The accuracy of an analytical method is defined as the proximity of the test findings obtained by the method to the true value. This means assessing the precision of the analytical procedure. The percent recovery is determined by the test of a known quantity of analyte within the linearity range.

1.6.1.2 Precision

We can define the precision of an analytical procedure as the closeness of agreement through multiple measurements acquired from repeated sampling of the same standardized sample under specified conditions. Should be examined utilizing homogeneous, authentic samples.

Expressed as Standard Deviation/ Relative Standard Deviation (SD/RSD): % RSD = (Standard Deviation / Mean) x 100

1.6.1.3 Specificity

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) defines assay specificity as the capability to reliably and precisely quantify the analyte amidst additional components that may be anticipated in the sample medium. The specific term often denotes a procedure that yields a response exclusively for a single analyte.

1.6.1.4 Selectivity

The selectivity of the method is its ability to detect the analyte among potential interfering components in the sample matrix. It is the capacity of a separative approach to distinguish between several substances. It quantifies the relative positional relationship between two peaks. This approach yields responses for several chemical entities that may or may not be isolated from one another. The determination is made by comparing the test findings of the analyte with and without the inclusion of potentially interfering substances.

1.6.1.5 Limit of Detection

The limit of detection (LOD) of an analytical process is the minimum quantity of an analyte in a sample that can be identified, albeit not necessarily quantified, under specified experimental conditions. It signifies that the sample is either below or above a specific threshold. The LOD will be contingent not only upon the analytical process but also on the type of instrument utilized.

1.6.1.6 Limit of Quantitation

The limit of quantitation (LOQ) is the minimum concentration of an analyte in a sample that can be quantitatively quantified with an acceptable degree of accuracy and precision under the specified operational circumstances of the method. The LOQ may differ based on the utilized methodology and the characteristics of the sample. It is typically employed for the identification of contaminants or degradation byproducts.

1.6.1.7 Linearity

Linearity refers to the method's capacity to yield test findings that are directly proportionate to the analyte concentration within a specified range. The linear relationship must be assessed over the entire span of the analytical method. A standard stock solution can be directly diluted to establish the drug substance. Linearity must be assessed through visual examination of a graph depicting concentration on the x-axis and mean response on the y-axis. Compute the regression equation, Y-intercept, and correlation coefficient. Data from the regression line may assist in providing mathematical estimates of the extent of linearity. A minimum of five concentrations is advised for the assessment of linearity.

1.6.1.8 Range

The range of an analytical technique is the interval between the higher and lower concentrations of the analyte in the sample, within which the procedure has been shown to possess adequate precision, accuracy, and linearity. Typically obtained from linearity experiments, the specific range is contingent upon the intended use of the process.

1.6.1.9 Robustness

This measure assesses an analytical method's ability to remain unaffected by minor, intentional procedural alterations, thereby indicating its variability under standard laboratory conditions.

1.6.1.10 Ruggedness

Degree of consistency of test results derived from examining the same sample under various standard testing circumstances. This includes factors such as the use of analysts, instruments, procedures, reagents, columns, and Thin Layer Chromatography (TLC) plates. That is, the absence of impact from environmental variables on the procedure. The comparison of test result reproducibility to assay precision serves as a direct indicator of the method's robustness.

1.6.1.11 System Suitability Testing

System suitability testing is an essential element in the majority of analytical methodologies. Upon validation of the method or system, the effort shifts to assessing the system's appropriateness for operation within the established boundaries. The assessments are founded on the premise that the apparatus, electronics, analytical procedures, and samples to be examined form a cohesive system that can be assessed as a whole. The parameters for the system suitability test to be defined for a specific method are contingent upon the nature of the procedure being verified.

1.7 Aim of this Work

The main objective of this study is:

To determine the quantity of different types of food additives in various types of commercial white flour.

The specific aims of this research are:

- To prepare the raw flour material by sieving it with a special sieve.
- To prepare different standard concentrations of each additive (PB, alloxan, and PI) mixed with pure flour.

- To construct calibration curves with simple linear equations for standard concentrations of additives depending on (ATR-FTIR) readings that determine their existence quantitatively.
- To estimate the concentrations of PB, alloxan, and PI in various types of commercial flours in the Palestinian market by using the (ATR-FTIR).

Chapter Two

2. Literature Review

2.1 Determination and Degradation of Potassium Bromate Content in Dough and Bread Samples

Abu-Obaid and colleagues (2016) determine PB levels in bread, noting that this flour improver, used for over 80 years as a maturation agent, is a potential carcinogen. Abu-Obaid, et al., developed a new rapid, simple, precise, and accurate testing method to determine PB levels in bread by reacting bromate ions with iodide ions in an acidic medium to produce iodine, which is measured by its absorbance at 352 nm, while bromate ions react with iodide during the first 3 minutes after the reaction begins. This method has been successfully applied to the determination of bromate ions in commercial bread samples. Furthermore, Abu-Obaid et al., found that bromate ions alone degraded at about 400°C, while they degrade at lower temperatures (150°C - 200°C) during bread making due to the presence of metals like Fe, Mg, Zn, Mn, Cu, and Al in flour acting as catalysts. It suggests that using two grams of PB per 60 kg bag of flour is safe. The research involved preparing standard solutions and testing bread samples with varying PB amounts, using UV spectrophotometry to measure iodine absorbance and calculate bromate concentrations.

2.2 Analysis of Potassium Bromate and Hydrocyanic Acid Contents of Bread and Wheat Flour Samples

Ojo and colleagues (2013) assessed the hydrocyanic acid and PB contents in 14 frequently consumed bread loaves and four prominent wheat flour brands in Nigeria, Karu, Nasarawa State, finding that all samples contained PB levels above the safe limit for human consumption, with differing quantities of hydrocyanic acid, which, nonetheless, will not result in the fatal dosage of

35 mg of hydrocyanic acid per kg of body weight. Potassium bromate levels in bread ranged from 0.5 µg/g to 8.4 µg/g, while in wheat flour samples, it ranged from 0.83 µg/g to 1.42 µg/g. Hydrocyanic acid content ranged from 0.706 to 1.498 mg/1000 g in wheat flour and 1.510 to 3.676 mg/1000 g in bread. Despite the ban by the National Agency for Food and Drug Administration and Control (NAFDAC) on the use of PB in baking due to its toxic effects, numerous Nigerian bakers persist in utilizing it as a dough conditioner and maturing agent to augment income. Ojo et al. emphasize the value of ongoing monitoring and implementation of the prohibition on PB utilization in the baking sector within the study area of NAFDAC**.**

2.3 First Report on the Presence of Alloxan in Bleached Flour by LC-MS/MS Method

Giaccone et al. (2017) set out to investigate the presence of alloxan in bread, pastry, and cake bleached flour to verify possible risks for consumers related to the use of chemicals for flour bleaching. Alloxan is one of the minor side products of oxidation that results after the chemical bleaching of wheat flour, when many chemical substances are used. Alloxan is known for its toxicological effects, including diabetogenic and carcinogenic actions, making its presence in food products a significant concern. A selective Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC MS/MS) method with precolumn derivatization was developed to detect alloxan in flour samples. This method was validated in accordance with ISO/IEC/EN 17025 for linearity, quantification limit, detection limit, precision, accuracy, and ruggedness determination. Around 175 flour samples were analyzed for alloxan determination. Acceptable outcomes were produced for the analyte, with an LOD of 0.73 mg kg^{-1}, an LOQ of 0.85 mg kg^{-1}, and recovery values between 94% and 102%. Analysis of samples from Sicily revealed alloxan trace levels in 24% of the samples, with mean values of 0.95 ± 0.04 mg kg^{-1}, suggesting that chemical bleaching agents like chlorine gas may produce alloxan as a by-product. The presence of alloxan was detected only in cake flour samples. Giaccone et al. call for further research with more samples to better understand the potential health risks associated with alloxan in bleached flour products.

2.4 Determination of Bromate and Iodate from Bread and Flour by Ion Chromatography

Chavan et al. (2019) analyze PB and PI, which are used as oxidizers in bread production to improve dough quality but have been banned or regulated in many countries due to health concerns, including their classification as potential carcinogens. For this reason, an accurate, sensitive, simple, and reproducible ion chromatography method was developed to determine bromate and iodate in bread and flour. Bromate and iodate were separated using a high-capacity anion exchange column by isocratic elution with a flow rate of 1.0 mL/min, and a substance called acidified promethazine was added as a post-column high-capacity ion reagent (PCR). It's important to note that the mobile phase was 20 mM sodium hydroxide, and the ion chromatographic PCR- UV detection technique was carried out. The linearity of the procedure has been tested for bromate in the range of 0.03 mg/l to 2.0 mg/l and for iodate in the range of 0.05 mg/l to 5.0 mg/l. Moreover,

a correlation coefficient (R^2) of more than 0.999 was shown for iodate and bromate. The method demonstrated excellent linearity, specificity, sensitivity, reproducibility, and ruggedness. The LOD and LOQ have also been decided for bromate as 0.01mg/l & 0.03mg/l and for iodate as 0.03 mg/l & 0.05 mg/l, respectively. That's why this method is suitable for regular analysis. Chavan and his colleagues utilized Thermo Fisher Dionex Ion Chromatography equipment with a VWD detector and specific reagents to achieve effective separation and detection of bromate and iodate at low concentrations.

2.5 Analysis of Potassium Iodate and Potassium Bromate in Bakery Products -by Electroanalytical Techniques

Duvvuri and Panchagnula (2016) analyze PI and PB in bakery products using electroanalytical techniques, specifically colorimetry, PB levels in bread were found to be between 4 and 5 mg/L. Bromate is used in the baking industry to improve flour quality and strengthen dough but poses health risks such as renal failure and cancer if not properly baked, leading to its ban in several countries. The methodology involved preparing bread samples from various outlets, creating a stock solution of PB, and using colorimetric measurements at 520 nm to determine bromate concentration. Results showed a linear relationship between concentration and absorbance, verifying Beer-Lambert's law, with the method proving accurate and reproducible compared to spectrophotometric analysis. The research concludes that the colorimetric method is precise, cost-effective, and less time-consuming for analyzing PB in bakery products.

Chapter Three

3. Materials and Methods

3.1 Chemicals

The chemicals used are potassium bromate, alloxan, and potassium iodate. All chemicals were purchased from Sigma-Aldrich. Wheat seeds were purchased from a local farmer in Abu-Dees, while various types of commercial flour were purchased from the Palestinian market. All samples were prepared by using mortar.

3.2 Instruments

The instruments used are a Bruker TENSOR II Infrared Spectrophotometer, outfitted with an ATR (Attenuated Total Reflectance) accessory, and an analytical balance (Hitachi).

Fig. 1: Bruker Tensor II device used in the study.

3.3 Experimental Part

3.3.1 Sieving Process

We obtained pure wheat flour without any additives, made directly from grinding wheat in a flour mill. Place the flour in the oven (100-120°C) to thoroughly dry it from any moisture. Next, the dried flour was sieved to remove the bran by using sieve shaker 425 µm and 250 µm sieves.

3.3.2 Standard Preparation

It is important to prepare different standard concentrations for each type of additive. A stock mixture of each additive was prepared by accurately measuring a specific mass of flour and the additives; then, each mixture was created by thoroughly combining the compounds using a mortar and pestle for at least half an hour to ensure the formation of homogeneous mixtures. Take 1.0000 ± 0.0001g of PB, then add 9.0000 ± 0.0001g of pure sieved flour to prepare a stock mixture with a concentration of 10% w/w and mix it using a mortar for 30 minutes. Different diluted mixtures were prepared according to the dilution law $M_1V_1=M_2V_2$ by using sieved pure flour for dilution.

Table 1: Standard concentrations of different types of additives.

Type of additive	Standard concentrations
Potassium bromate	Pure flour (0), 12.5, 25, 50, 75, 90, 100 mg/kg
Alloxan	Pure flour (0), 0.25, 0. 5, 0.95, 1.5, 2 mg/kg
Potassium iodate	Pure flour (0), 12.5, 25, 50, 75, 90, 100 mg/kg

The same procedure was carried out to prepare the stock mixture and standard concentrations for both PI and alloxan, as in Table 1.

3.3.3 Mixtures for Calibration Curve

Mixtures for constructing the calibration curves for quantitative analysis of PB, alloxan, and PI are shown in Table 1. The concentration of PB and PI in these mixtures ranges from 0 to 100 mg/kg, since alloxan concentrations in these mixtures range from 0.0 to 2 mg/kg. The prepared sample shown in Table 1 was scanned from 4000 to 400 cm^{-1} by using ATR-FTIR. The spectrum measured was collected as data points.

3.3.4 FTIR Analysis

FTIR is an effective tool for chemical identification because each molecule produces a distinct spectral pattern. It is important to note that each run was scanned 200 times.

Chapter Four

4. Results and Discussion

4.1. IR Spectra of Flour Samples and Other Additives

The IR spectra of flour, PB, alloxan, and PI in this study in terms of the wavenumbers of the absorption peaks are illustrated in figures 2, 3, 4, and 5 (it is important to note that each value is the average of three reading at three different times). From the spectra of IR, the most important peaks used for analysis of flour are 758 cm^{-1}, 852.2 cm^{-1}, and 930.8 cm^{-1}, which all indicate the FP region; 1076.4 cm^{-1} reflects the FP region and C–O (ether, ester, alcohol); 1540.3 cm^{-1} and 1645.9 cm^{-1} indicates C=C (alkene). While the most important peaks used for analysis of PB are 1216.3 cm^{-1} and 1366.2 cm^{-1}, both reflect the FP region. Furthermore, the most important peaks used for analysis of alloxan are 411.1 cm^{-1} and 695.2 cm^{-1}, both of which indicate the FP region. Finally, the most important peak used for analysis of PI is 1216.3 cm^{-1}, which indicates the FP region.

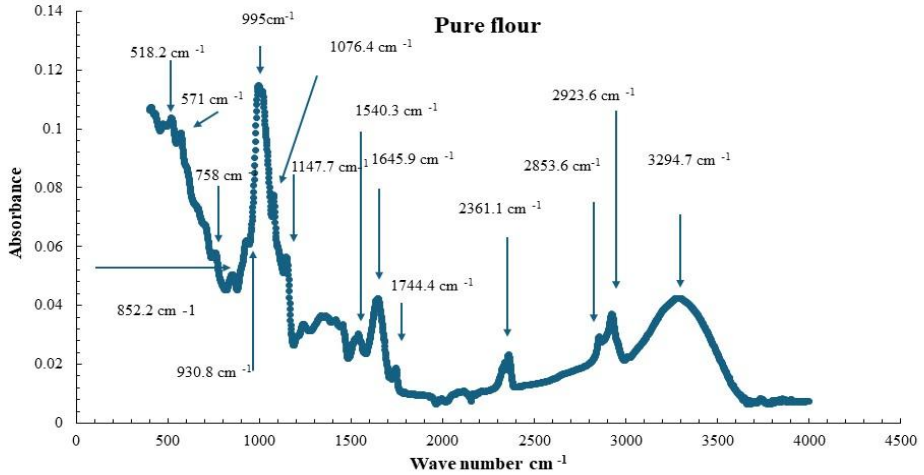

Fig. 2: IR spectra of flour used in this study.

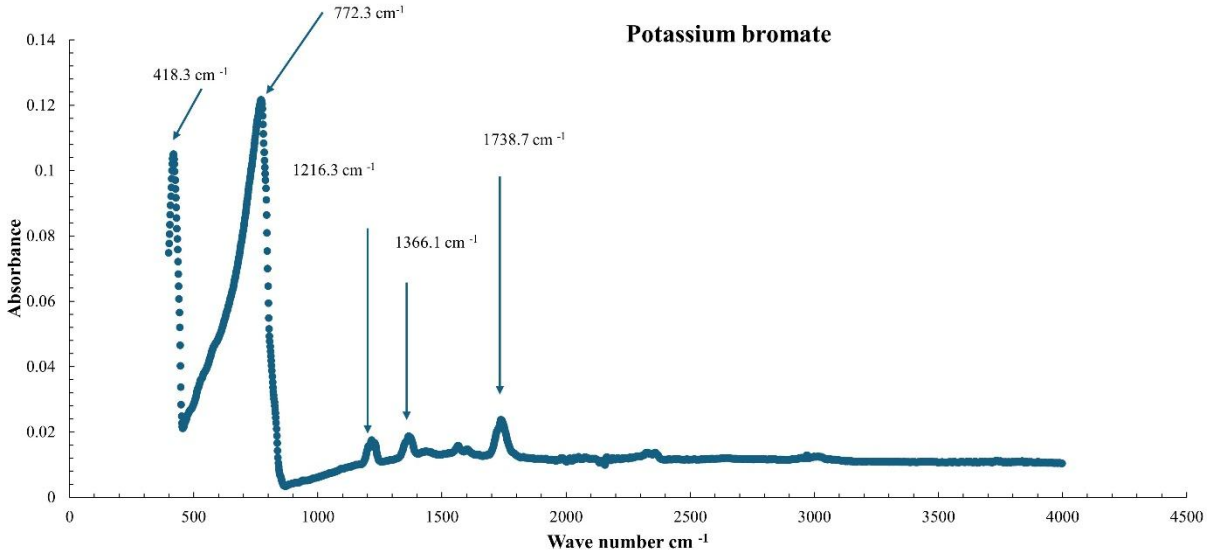

Fig. 3: IR spectra of PB used in this study.

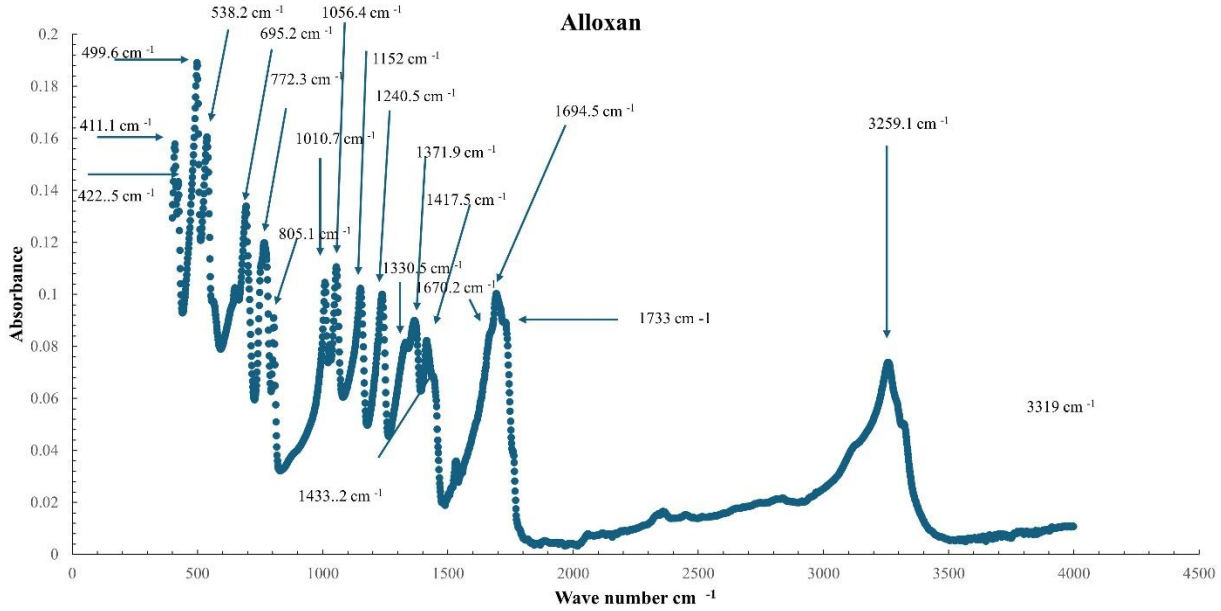

Fig. 4: IR spectra of alloxan used in this study.

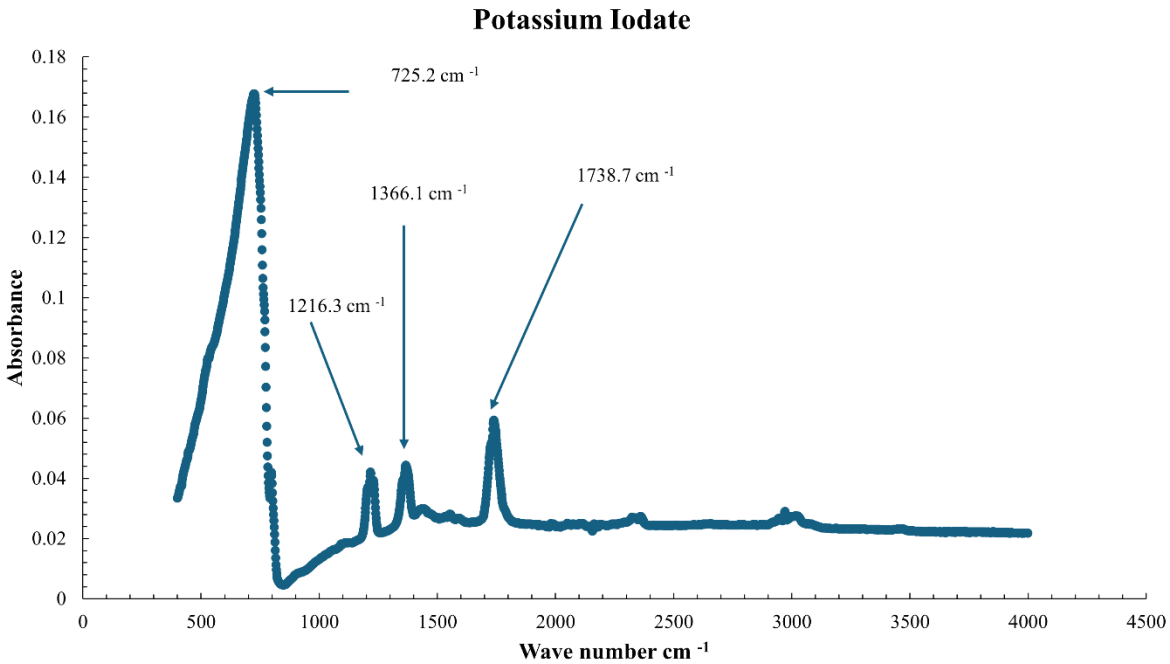

Fig. 5: IR spectra of PI used in this study.

4.2 The Criteria for Determining the Presence of Additives in Flour

This research employs absorbance ratios at two wavenumbers in the IR spectrum to verify the existence of additives in flour because the absorbance at specific wavenumbers for particular materials may exhibit slight variations over time and among instruments, contingent upon the measurement technique utilized for the IR spectrum (ATR, liquid film, CCl_4 solution, KBr, etc.). Consequently, this study endorses the absorbance ratio, rather than the absolute absorbance at a specific wavenumber, as a normalizing method to eliminate any variable that could alter the absorbance readings at a particular wavenumber.

4.3 Constructing Calibration Curves for Quantitative Determination of Additives in Four

The quantitative analysis of flour containing different types of additives is based on the absorbance ratio of each additive to pure flour at two specific wavenumbers as a function of the concentration of a certain additive in the pure flour. A multitude of calibration curves was generated, but only those with a coefficient of determination (R^2) exceeding 0.9 were accepted. We build a calibration curve to determine the quantitative analysis of the additives.

4.3.1 Constructing Calibration Curves for Quantitative Determination of Potassium Bromate in Four.

The absorbance of flour spiked with PB, where the concentration of PB ranges from 0.0 to 100 ppm, at specific wavenumbers, together with the absorbance ratios, is shown in Table 2. These absorbance ratios as a function of PB concentration in ppm were plotted to generate calibration curves, enabling quantitative analysis, as illustrated in Figure 6. The calibration curves derived from Figure 6 are encapsulated in Table 3. These calibration curves collectively serve as a reliable instrument for the quantitative analysis of PB within pure flour samples.

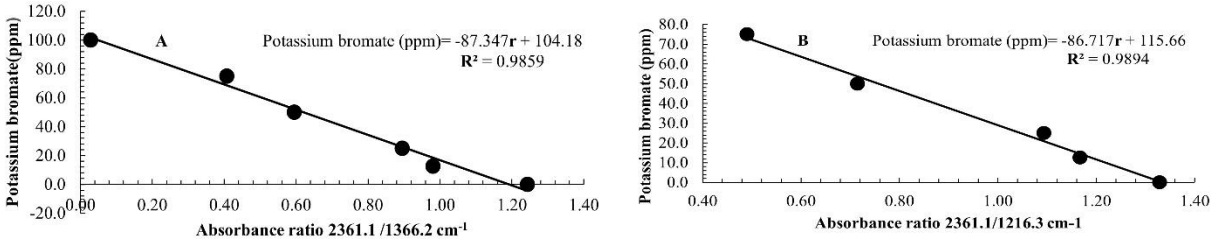

Fig. 6 (A, B): Calibration curve equations, by which quantitative determination of PB in flour can be potentially calculated. Data for these curves are obtained from Table 2.

Table 2: The absorbance of PB in flour as a function of concentration (ppm) and the corresponding wave numbers, along with absorbance ratios relative to pure flour at each wave number.

Concentration of PB (ppm)	Absorbance at 2361.1 cm^{-1}	Absorbance at 1366.2 cm^{-1}	Absorbance ratio 2361.1/1366 .2	Absorbance at 1216.3 cm^{-1}	Absorbance ratio 2361.1/1216.3
0	0.0232	0.0187	1.2433	0.0175	1.3286
12.5	0.0236	0.0241	0.9811	0.0202	1.1673
25	0.0229	0.0255	0.8955	0.0209	1.0935
50	0.0214	0.0360	0.5949	0.0299	0.7149
75	0.0127	0.0313	0.4073	0.0260	0.4907
90	0.0214	0.0360	0.5949	0.0299	0.7149
100	0.0197	0.6790	0.0290	0.0292	0.6741

Table 3: Calibration curve equations, by which quantitative determination of PB in flour can be potentially computed.

Equation No	Equation	Equation's details
1	Potassium bromate (ppm) = -87.347**r** + 104.18 $\mathbf{R^2}$ = 0.9859.	Derived by calculating the absorbance ratio **r** (absorbance of the flour sample at wavenumber 2361.1 cm^{-1}divided by absorbance of the flour sample at 1366.2 cm^{-1}). As illustrated in Fig. 6. A.
2	Potassium bromate (ppm) = -86.717**r**+ 115.66 $\mathbf{R^2}$ = 0.9894.	Derived by calculating the absorbance ratio **r** (absorbance of the flour sample at wavenumber 2361.1 cm^{-1}divided by absorbance of the flour sample at1216.3 cm^{-1}). As illustrated in Fig. 6. B.

4.3.2 Constructing Calibration Curves for Quantitative Determination of Alloxan in Flour

The absorbance of flour spiked with alloxan, where the concentration of alloxan ranges from 0.0 to 2 ppm, at specific wavenumbers, together with the absorbance ratios, is shown in Table 4. These absorbance ratios as a function of alloxan concentration in ppm were plotted to generate calibration curves, enabling quantitative analysis, as illustrated in Figure 7. The calibration curves derived from Figure 7 are encapsulated in Table 5. These calibration curves collectively serve as a reliable instrument for the quantitative analysis of alloxan within pure flour samples.

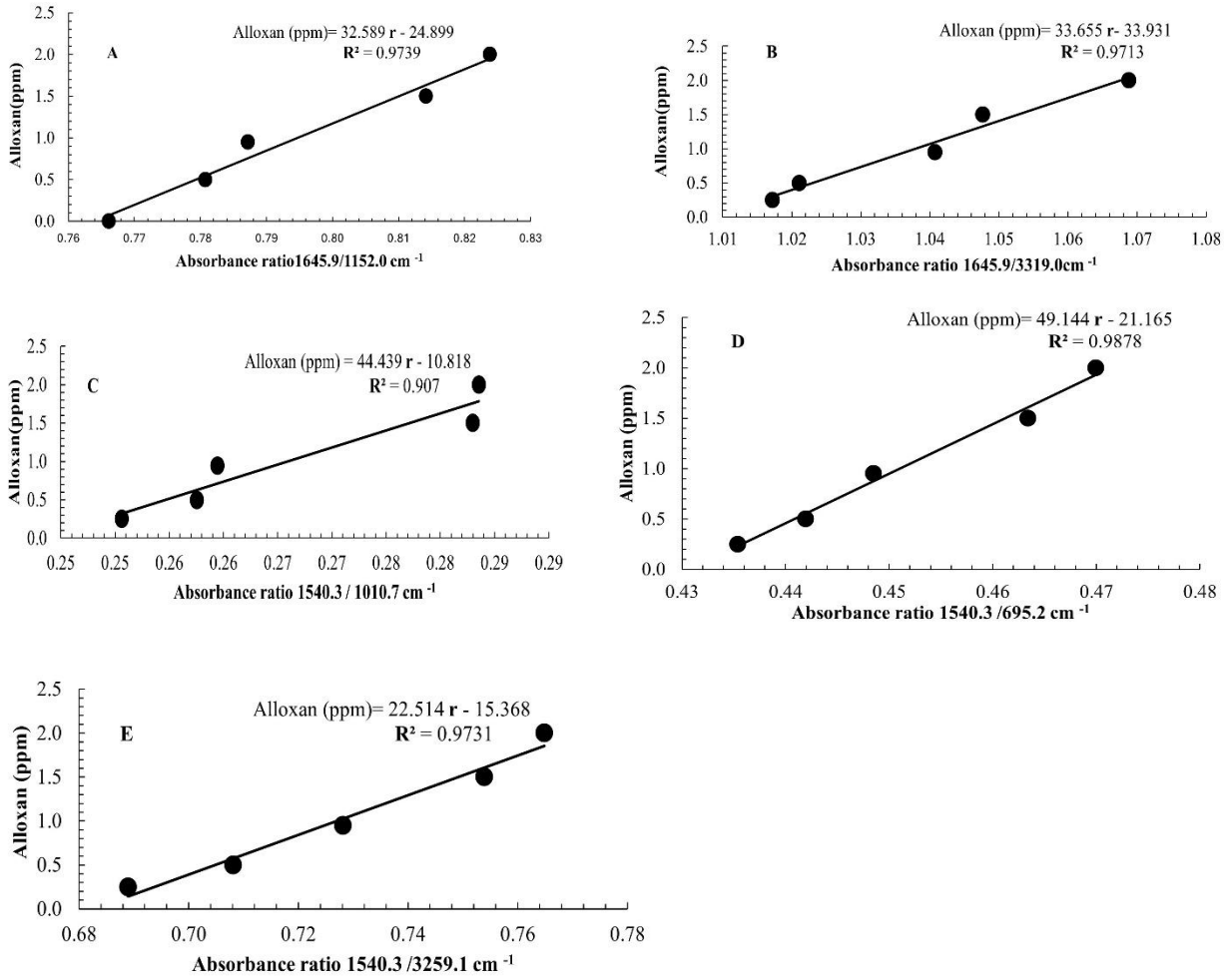

Fig 7 (A, B, C, D, E)**:** Calibration curve equations, by which quantitative determination of alloxan in flour can be potentially calculated. Data for these curves are obtained from Table 4.

Table 4: The absorbance of alloxan in flour as a function of concentration (ppm) and the corresponding wave numbers, along with absorbance ratios relative to pure flour at each wave number.

Concentration of alloxan (ppm)	Absorbance at 1645.9 cm^{-1}	Absorbance at 1152.0 cm^{-1}	Absorbance ratio 1645.9/1152.0	Absorbance at 3319.0 cm^{-1}	Absorbance ratio 1645.9/3319.0	Absorbance at 1540.3 cm^{-1}	Absorbance at 1010.7 cm^{-1}	Absorbance ratio 1540.3/1010.7	Absorbance at 695.2cm^{-1}	Absorbance ratio 1540.3/695.2	Absorbance at 3259.1 cm^{-1}	Absorbance ratio 1540.3/3259.1
0	0.0426	0.0554	0.7661	0.0416	1.0209	0.0305	0.1128	0.2706	0.0677	0.4510	0.0.0416	0.7330
0.25	0.0479	0.0609	0.7862	0.0471	1.0172	0.0327	0.1287	0.2506	0.0741	0.4354	0.0468	0.689
0.5	0.0423	0.0542	0.7807	0.0415	1.0211	0.0293	0.1138	0.2576	0.0663	0.4419	0.0414	0.7081
0.95	0.0510	0.0648	0.7872	0.0490	1.0407	0.0357	0.1376	0.2594	0.0796	0.4485	0.049	0.7282
1.5	0.0386	0.0474	0.8142	0.0369	1.0477	0.0278	0.0982	0.2830	0.06	0.4634	0.0369	0.7539
2	0.0474	0.0575	0.8238	0.0443	1.0688	0.0339	0.1195	0.2836	0.0721	0.47	0.0443	0.7649

Table 5**:** Calibration curve equations, by which quantitative determination of alloxan in flour can be potentially computed.

Equation No	Equation	Equation's details
1	Alloxan (ppm) = 32.589 **r** - 24.899 $\mathbf{R^2}$ = 0.9739.	Derived by calculating the absorbance ratio **r** (absorbance of the flour sample at wavenumber 1645.9 cm^{-1} divided by absorbance of the flour sample at 1152.0 cm^{-1}). As illustrated in Fig. 7. A.
2	Alloxan (ppm) = 33.655**r** - 33.931 $\mathbf{R^2}$ = 0.9713.	Derived by calculating the absorbance ratio **r** (absorbance of the flour sample at wavenumber 1645.9 cm^{-1} divided by absorbance of the flour sample at 3319.0 cm^{-1}). As illustrated in Fig. 7. B.
3	Alloxan (ppm) = 44.439 **r** - 10.818 $\mathbf{R^2}$ =0.907.	Derived by calculating the absorbance ratio **r** (absorbance of the flour sample at wavenumber1540.3 cm^{-1} divided by absorbance of the flour sample at 1010.7 cm^{-1}). As illustrated in Fig. 7. C.
4	Alloxan (ppm) = 49.144**r** - 21.165 $\mathbf{R^2}$ = 0.9878.	Derived by calculating the absorbance ratio **r** (absorbance of the flour sample at wavenumber1540.3 cm^{-1} divided by absorbance of the flour sample at 695.2 cm^{-1}). As illustrated in Fig. 7. D.
5	Alloxan(ppm) = 22.514**r** -15.368 $\mathbf{R^2}$ = 0.9731.	Derived by calculating the absorbance ratio **r** (absorbance of the flour sample at wavenumber1540.3 cm^{-1} divided by absorbance of the flour sample at 3259.1 cm^{-1}). As illustrated in Fig. 7. E.

4.3.3 Constructing Calibration Curves for Quantitative Determination of Potassium Iodate in Flour

The absorbance of flour spiked with PI, where the concentration of PI ranges from 0.0 to 100 ppm, at specific wavenumbers, together with the absorbance ratios, is shown in Table 6. These absorbance ratios as a function of PI concentration in ppm were plotted to generate calibration curves, enabling quantitative analysis, as illustrated in Figure 8. The calibration curves derived from Figure 8 are encapsulated in Table 7. These calibration curves collectively serve as a reliable instrument for the quantitative analysis of PI within pure flour samples.

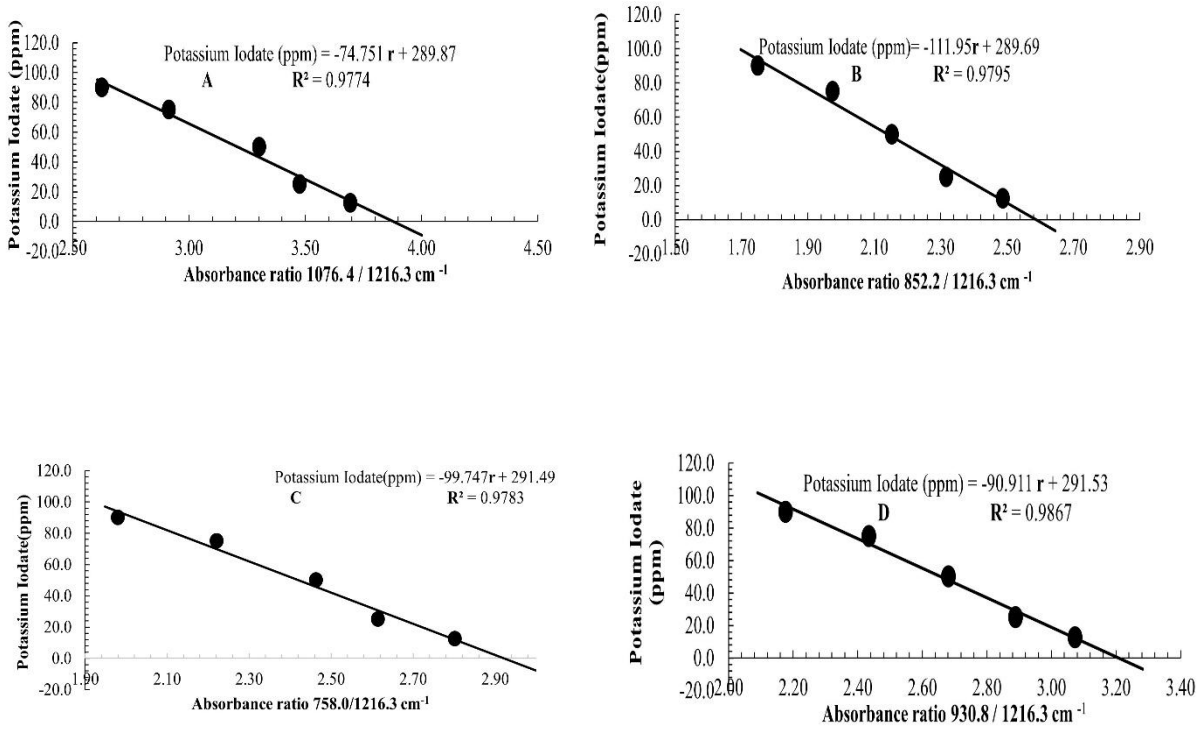

Fig. 8 (A, B, C, D)**:** Calibration curve equations, by which quantitative determination of PI in flour can be potentially calculated. Data for these curves are obtained from Table 6.

Table 6: The absorbance of PI in flour as a function of concentration (ppm) and the corresponding wave numbers, along with absorbance ratios relative to pure flour for each wave number.

Concentration of PI (ppm)	Absorbance at 1076.4 cm^{-1}	Absorbance at 1216.3 cm^{-1}	Absorbance ratio 1076.4/1216.3	Absorbance at 852.2 cm^{-1}	Absorbance ratio 852.2/1216.3	Absorbance at 758.0 cm^{-1}	Absorbance ratio 758.0/1216.3	Absorbance at 930.8 cm^{-1}	Absorbance ratio 930.8/1216.3
0	0.0775	0.0298	2.6050	0.0505	1.6972	0.0580	1.9489	0.0622	2.0892
12.5	0.0800	0.0217	3.6946	0.0539	2.4881	0.0607	2.8018	0.0666	3.0736
25	0.0819	0.0235	3.4766	0.0546	2.3173	0.0615	2.6140	0.0680	2.890
50	0.0959	0.0290	3.3015	0.0626	2.1533	0.0716	2.4635	0.0779	2.6812
75	0.0649	0.0223	2.9135	0.0440	1.9749	0.0495	2.2208	0.0542	2.4350
90	0.0800	0.0305	2.6248	0.0533	1.7494	0.0604	1.9801	0.0664	2.1767
100	0.0810	0.0202	4.0027	0.0536	2.6464	0.0607	3.0011	0.0664	3.2831

Table 7**:** Calibration curve equations, by which quantitative determination of PI in flour can be potentially computed.

Equation No	Equation	Equation's details
1	Potassium Iodate (ppm) = -74.751**r** +289.87 **R^2** = 0.9774.	Derived by calculating the absorbance ratio **r** (absorbance of the flour sample at wavenumber 1076.4 cm^{-1} divided by absorbance of the flour sample at 1216.3 cm^{-1}). As illustrated in Fig. 8. A.
2	Potassium Iodate (ppm)=-111.95**r** +289.69 **R^2** = 0.9795.	Derived by calculating the absorbance ratio **r** (absorbance of the flour sample at wavenumber 852.2 cm^{-1}divided by absorbance of the flour sample at 1216.3 cm^{-1}). As illustrated in Fig. 8. B.
3	Potassium Iodate (ppm) = -90.911**r** + 291.53 **R^2** = 0.9867.	Derived by calculating the absorbance ratio **r** (absorbance of the flour sample at wavenumber758.0 cm^{-1}divided by absorbance of the flour sample at 1216.3 cm^{-1}). As illustrated in Fig. 8. C.
4	Potassium Iodate (ppm) = -90.911 **r** + 291.53 **R^2** = 0.9867.	Derived by calculating the absorbance ratio **r** (absorbance of the flour sample at wavenumber 930.8 cm^{-1} divided by absorbance of the flour sample at 1216.3 cm^{-1}). As illustrated in Fig. 8. D.

It is shown from our equations constructed for quantitative analysis of different additives in flour that R^2 sometimes is small, about 0.90, but in most cases exceeds 0.97. This is because the work was conducted with a solid heterogeneous mixture, making it very challenging to achieve a homogeneous mixture. Mixing was kept for about half an hour, but even though a 100% homogeneous mixture was not attained, only a 97% or 98% homogeneous mixture was achieved, so the calibration curve was difficult to have R^2 0.99.

4.4 Quantitative Analysis of Additives (Potassium Bromate, Alloxan, and Potassium Iodate) in Different Types of Commercial Flour in the Palestinian Market.

As mentioned earlier, the best calibration curves with R^2 more than 0.90 were considered. The calibration curve was built to perform quantitative analysis of several types of additives in different types of commercial flour in the Palestinian market (because bread is considered one of the most popular food items consumed among people). By applying the absorbance ratio at certain two wavenumbers in the equation of the best calibration curve with R^2 more than 0.90, the quantitative analysis of additives in different types of commercial flour was obtained as follows:

4.4.1 Quantitative Analysis of Potassium Bromate in Different Types of Commercial Flour in the Palestinian Market.

Potassium bromate was detected in all analyzed flour samples in the Palestinian market. A careful examination of Table 8 reveals that the estimated amounts of PB in each sample, derived from equations 1 and 2, are very similar, confirming the applicability and accuracy of our method. Furthermore, the accepted amount of PB in flour does not exceed 50 mg/kg in most countries' regulations. Although our results indicated that the estimated amount of PB is sometimes slightly below the accepted level and at other times slightly above, all measurements remain within this acceptable range. However, this small excess amount in certain samples must be considered when dealing with the health of human beings because PB is a carcinogenic factor and has many negative effects on our health. Therefore, this small increase should not be allowed, and the relevant authorities must accurately determine the amount of bromate in flour.

Table 8: The absorbance of PB in flour as a function of concentration (ppm) and the corresponding wave numbers, along with absorbance ratios relative to commercial flour at each wave number.

Type of flour	Absorbance at 2361.1cm^{-1}	Absorbance at 1366.2 cm^{-1}	Absorbance ratio 2361.1/1366.2	Concentration of PB in flour based on eq 1	Absorbance at 1216.3cm-1	Absorbance ratio 2361.1/1216.3	Concentration of PB in flour based on eq 2
A	0.0287	0.0528	0.5431	56.8	0.0390	0.7340	52.0
B	0.0177	0.0406	0.4364	66.1	0.0293	0.6033	63.3
C	0.0276	0.0404	0.6834	44.5	0.0301	0.9175	36.1
D	0.0223	0.0554	0.4020	69.1	0.0415	0.5367	69.1
E	0.0269	0.0593	0.4536	64.6	0.0438	0.6145	62.4
F	0.0240	0.0529	0.4540	64.5	0.0391	0.6152	62.3
G	0.0251	0.0364	0.6901	43.9	0.0277	0.9087	36.9

4.4.2 Quantitative Analysis of Alloxan in Different Types of Commercial Flour in the Palestinian Market.

It should be noted again that alloxan is not added to flour; it's a byproduct of thermal and chemical treatment of flour. Alloxan is harmful to human health because it negatively affects pancreatic cells, the malfunction of which leads to diabetes mellitus type 1. Therefore, the concentration of alloxan should not be detected at all in the commercial flour. This aligns with the results of this work, as shown in Table 9, where six of seven samples (A, B, C, D, E, and G) showed no detectable alloxan, falling below the detection limit (this is expected because, as mentioned above, alloxan is not added but is produced by thermal and chemical treatment of flour). Furthermore, all five equations have very close results; this confirms that our calibration curves are applicable for the determination of alloxan in flour. That's why the method is applicable and trusted. On the other hand, alloxan was detected in sample F, with a concentration of 1.6281 ppm. This type of flour is used in making cakes, as it requires a lot of thermal and chemical treatments to give it a spongy texture. Therefore, it is expected that it will contain alloxan. Although this type of flour provides an ideal texture for cakes, it is harmful to human health. Therefore, it is recommended to avoid using this type of flour.

It is important to note that research reveals that alloxan was detected in cake flour, associated with the application of bleaching agents (chemical oxidizers) such as chlorine dioxide and chlorine gas in the manufacturing of fine and smooth cake flour. These chemicals modify the protein structure of the flour by tightening the protein molecules, which increases the flour's capacity to absorb sugar and fat, thereby enhancing its baking performance. Furthermore, these chemicals facilitate the rapid aging of flour, substituting natural aging mechanisms. Moreover, chlorine gas can chemically interact with some types of proteins in the flour, such as gluten, producing alloxan as a by-product. High-gluten cake flour may possess 5 to 8 g of gluten per 100 g, indicating a potential relationship between chlorine exposure and the synthesis of alloxan (Giaccone et al,. 2017).

Table 9: The absorbance of alloxan in flour as a function of concentration (ppm) and the corresponding wave numbers, along with absorbance ratios relative to commercial flour for each wave number

Type of flour	Absorbance at 1645.9cm^{-1}	Absorbance at 1152.0 cm^{-1}	Absorbance ratio 1645.9/1152.0	Concentration of alloxan in flour based on eq 1	Absorbance at 3319.0 cm^{-1}	Absorbance ratio 1645.9/3319.0	Concentration of alloxan in flour based on eq 2	Absorbance at 1540.3 cm^{-1}	Absorbance at 1010.7 cm^{-1}	Absorbance ratio 1540.3/1010.7	Concentration of alloxan in flour based on eq3	Absorbance at 695.2 cm^{-1}	Absorbance ratio 1540.3/695.2	Concentration of alloxan in flour based on eq 4	Absorbance at 3259.1 cm^{-1}	Absorbance ratio 1540.3/3259.1	Concentration of alloxan in flour based on eq 5
A	0.0640	0.0892	0.7716	0.2459	0.0658	0.9718	Not detected	0.0426	0.1914	0.2224	Not detected	0.1053	0.4042	Not detected	0.0664	0.6411	Not detected
B	0.0475	0.0634	0.7493	Not detected	0.0502	0.9465	Not detected	0.0322	0.1436	0.2242	Not detected	0.0801	0.4012	Not detected	0.0503	0.6393	Not detected
C	0.0469	0.0644	0.7289	Not detected	0.0467	1.0053	Not detected	0.0316	0.1473	0.2143	Not detected	0.0837	0.3773	Not detected	0.0472	0.6691	Not detected
D	0.0355	0.0484	0.7350	Not detected	0.0353	1.0079	Not detected	0.0231	0.1123	0.2059	Not detected	0.0642	0.3600	Not detected	0.0356	0.6491	Not detected
E	0.0732	0.0931	0.7861	0.7191	0.0739	0.9910	Not detected	0.0496	0.2175	0.2281	Not detected	0.1159	0.4280	Not detected	0.0744	0.6673	Not detected
F	0.0667	0.0819	0.8140	1.6281	0.0661	1.0081	Not detected	0.0450	0.1855	0.2427	Not detected	0.1034	0.4357	0.2487	0.0666	0.6762	Not detected
G	0.0426	0.0564	0.7562	Not detected	0.0424	1.0052	Not detected	0.0275	0.1273	0.2162	Not detected	0.0729	0.3777	Not detected	0.0428	0.6436	Not detected

4.4.3 Quantitative Analysis of Potassium Iodate in Different Types of Commercial Flour in the Palestinian Market

Potassium iodate was found in all studied samples of flour in the Palestinian market. A close examination of Table 10 reveals that the estimated amounts of PI in each sample, calculated using equations 1, 2, 3, and 4, are very similar, which confirms the applicability and accuracy of our method. Even though there is no agreement on the accepted amount of PI, its amount, according to regulations in most countries, should not exceed 45–75 mg/kg. Although our results indicated that the estimated amount of PI occasionally exceeds the accepted range, it remains within the limits set by regulations. It's odd that there's no agreement on the amount of PI in flour, as it's less harmful than PB. It is possible to take more PI, and it will not harm human health, but it affects those with thyroid problems. Therefore, it is recommended to impose more restrictions on the amount of PI in flour.

Table 10: The absorbance of PI in flour as a function of concentration (ppm) and the corresponding wave numbers, along with absorbance ratios relative to commercial flour for each wave number.

Type of flour	Absorbance 1076.4 cm^{-1}	Absorbance at 1216.3 cm^{-1}	Absorbance ratio 1076.4/1216.3	Concentration of PI in flour based on eq 1	Absorbance at 852.2 cm^{-1}	Absorbance ratio 852.2/1216.3	Concentration of PI in flour based on eq 2	Absorbance at 758.0 cm^{-1}	Absorbance ratio 758.0/1216.3	Concentration of PI in flour based on eq 3	Absorbance at 930.8cm^{-1}	Absorbance ratio 930.8/1216.3	Concentration of PI in flour based on eq 4
A	0.1177	0.0390	3.0164	64.4	0.0753	1.9277	73.9	0.0904	2.3157	60.5	0.0938	2.4041	73.0
B	0.0890	0.0293	3.0337	63.1	0.0587	1.9988	65.9	0.0699	2.3811	54.0	0.0724	2.4664	67.3
C	0.0899	0.0301	2.9840	66.8	0.0609	2.0220	63.3	0.0710	2.3590	56.2	0.0747	2.4809	66.0
D	0.0698	0.0238	2.9271	71.1	0.0462	1.9394	72.6	0.0545	2.2836	63.7	0.0571	2.3940	73.9
E	0.1319	0.0438	3.0118	64.7	0.0836	1.9101	75.9	0.0998	2.2795	64.1	0.1049	2.3965	73.7
F	0.1149	0.0391	9.9405	70.1	0.0744	1.9051	76.4	0.0893	2.2867	63.4	0.0924	2.3665	76.4
G	0.0793	0.0277	2.8670	75.6	0.0530	1.9174	75.0	0.0624	2.2571	66.4	0.0656	2.3710	76.0

4.5 Advantages of Our Method Compared to Other Methods

The most common method to determine the amount of alloxan is using High-Performance Liquid Chromatography (HPLC), with detectors based on measuring fluorescence. Alloxan should be reacted with phenylene diamine to create a fluorophore complex; therefore, only HPLC devices equipped with a fluorescence detector can analyze and validate the amount of alloxan. After extracting alloxan from flour, it should be reacted with phenylene diamine to produce alloxazine, which is then analyzed by HPLC to determine the amount of alloxan, as alloxan alone cannot be detected by HPLC. Although this method is very accurate, it is not considered simple and available because it needs a special type of HPLC and needs extraction of alloxan, sample preparation, organic synthesis, and chemical reaction, while our method is effortless; it only puts a small amount of flour on the device and directly measures by using mathematical equations derived from calibration curves.

The conventional method for analyzing PB and PI is based on oxidation-reduction titrations, but this method is not so applicable because it needs sample preparation, takes a long period of time, has a low detection limit, and is not accurate because there are many ingredients in flour (such as sugar) that interfere in oxidation-reduction reactions and may change the results appreciably. To perform an analysis based on oxidation-reduction, it is necessary to separate bromate and iodate from the flour before conducting the titration, which is a time-consuming process. Furthermore, the other methods for analyzing PB and PI are also time-consuming and require sample preparation, making them harder than our method.

On the other hand, FTIR is an analytical method employed to identify polymeric, organic, and some inorganic substances. It employs infrared light to analyze samples and examine their chemical characteristics. The main principle of FTIR is based on the interaction of IR radiation with matter, which allows for the determination of molecular structures. An FTIR spectrometer consists of a source, interferometer, sample compartment, detector, amplifier, analog-to-digital (A/D) converter, and a computer. The source generates radiation, which passes the sample through the interferometer and reaches the detector. Then the signal is amplified and converted to a digital signal by the amplifier and (A/D) converter, respectively. Eventually, the signal is transferred to a computer in which Fourier transform is carried out. Furthermore, FTIR is used for quantitative and qualitative analysis of additives in flour because of several advantages, such as:

1. Rapid analysis: FTIR is a fast analytical technique that allows for the quick assessment of flour samples. Traditional methods of analyzing additives can be time-consuming, requiring extensive sample preparation and lengthy procedures. In contrast, FTIR can provide results in a matter of minutes, making it suitable for high-throughput environments.

2. Minimal sample preparation: One of the significant advantages of FTIR is that it requires little to no sample preparation. This characteristic simplifies the analysis process and reduces the risk of contamination or alteration of the sample, which can occur with more invasive methods.

3. Non-destructive testing: FTIR is a non-destructive technique, meaning that it does not alter or consume the flour sample during analysis. This feature allows for further testing or use of the same sample after FTIR analysis, which is particularly beneficial when dealing with limited quantities of high-value samples.

4. Comprehensive information: FTIR provides qualitative information about various functional groups present in additives in the flour. By analyzing the absorption spectra, researchers can identify specific chemical bonds and functional groups associated with different additives, such as emulsifiers, preservatives, or colorants.

5. Environmentally friendly: The FTIR technique is considered environmentally friendly as it often eliminates the need for hazardous solvents and reagents typically used in traditional chemical analyses. This aspect aligns with modern laboratory practices aimed at reducing environmental impact.

6. High sensitivity and specificity: FTIR spectroscopy offers high sensitivity to detect even trace amounts of additives in flour samples. The specificity of FTIR allows for distinguishing between different types of additives based on their unique spectral fingerprints.

7. Versatility across different matrices: FTIR can be applied to various types of flour and their respective additives without significant modifications to the method. This versatility makes it an attractive option for quality control across different flour products.

Moreover, FTIR spectrometers are the third-generation IR spectrometers and have several prominent advantages: (1) The signal-to-noise ratio of the spectrum is significantly higher than the previous one. (2) The accuracy of avenumber is high. The error is within the range of ± 0.01 cm^{-1}. (3) The scan time of all frequencies is short (approximately 1 s). (4) The resolution is extremely high (0.1 ~ 0.005 cm^{-1}). (5) The scan range is wide (1000 ~ 10 cm^{-1}). (6) The interference from stray light is reduced. Due to these advantages, FTIR spectrometers have replaced dispersive IR spectrometers.

Therefore, FTIR is considered an excellent technique for detecting additives in flour.

4.6 Recommendations

Although the additives give many positive features to flour, such as whiteness, texture, and puffiness, they also have many negative effects on human health, as mentioned previously. The results of this study found that flour available in the Palestinian market contains these additives. To protect people's health, we are heading to the quality control and supervision department and concerned parties of the Palestinian National Authority to pay more attention to this issue, aiming to limit the use of these additives or limit their use to levels permitted internationally. These goals can be achieved by conducting the necessary tests on flour produced for the market and requiring flour factories to display labels detailing the ingredients of flour, additives, and other ingredients, as well as their quantities.

Chapter Five

5. Conclusion

This work established the efficacy of ATR-FTIR spectroscopy as a simple, rapid, accurate, robust, straightforward, and cost-consuming method without sample preparation for the quantitative assessment of PB, alloxan, and PI in white flour. Specific infrared absorption bands were effectively identified for each type of additive, resulting in the development of linear calibration curves that were accurate, reproducible, and precise.

The application of this method on commercial flour samples from the Palestinian market showed different concentrations of the examined additives, emphasizing the importance of regular monitoring to guarantee compliance with food safety rules and decrease potential health concerns. The results confirm that ATR-FTIR spectroscopy can function as a practical, viable substitute for more complicated and time-consuming analytical methods.

In summary, the devised technology presents a cost-effective and environmentally friendly solution suitable for standard quality control and regulatory surveillance of flour additives. Further research is recommended to extend its application to various food products and to improve its sensitivity for trace-level detection.

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التحليل الكمي لبرومات البوتاسيوم ويودات البوتاسيوم والألوكسان في الدقيق الأبيض باستخدام تقنية الانعكاس الكلي المخفف – مطيافية الأشعة تحت الحمراء بتحويل فورييه (ATR–FTIR)

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الملخص:

يعدّ القمح من محاصيل الحبوب بالغة الأهمية في العالم، وجزءا أساسيا من نظامنا الغذائي اليومي كمصدر غذائي رئيسي.حيث توجد أنواع مختلفة من القمح، مثلTriticum aestivum ، وهو المصدر الرئيسي للدقيق المستخدم في صناعة الخبز. على الرغم من غنى حبوب القمح الكاملة بالعناصر المفيدة، إلا أن الدقيق المصنوع منها يفتقر إلى الخصائص الريولوجية المرغوبة؛ لذا، من المهم إضافة بعض المواد لتحسين خصائصه. لسوء الحظ، أثارت المواد الكيميائية المضافة إلى دقيق القمح، وخاصة عوامل الأكسدة والتبييض، مخاوف متزايدة بشأن سلامة الغذاء وصحة الإنسان. تهدف هذه الدراسة إلى تحديد كمية برومات البوتاسيوم، والألوكسان، ويودات البوتاسيوم في الدقيق الأبيض باستخدام مطيافية الأشعة تحت الحمراء بتحويل فورييه والانعكاس الكلي المخفف(ATR–FTIR). تم تحضير مخاليط دقيق قياسية تحتوي على تراكيز مختلفة من كل مادة مضافة، وتم تحليلها لتحديد نطاقات الامتصاص المميزة. تم إنشاء منحنيات معايرة بناءً على نسب امتصاص الأشعة تحت الحمراء المختارة، مما مكّن من التحديد الكمي لكل مادة مضافة في مصفوفات الدقيق باستخدام المعادلات التالية:

برومات البوتاسيوم	$R^2 = 0.9894$	Potassium bromate (ppm) = -86.717r + 115.66
ألوكسان	$R^2 = 0.987$	Alloxan (ppm) = 49.144r - 21.165
يودات البوتاسيوم	$R^2 = 0.986$	Potassium Iodate (ppm) = -90.911r + 291.5

يمثل المتغير r نسبة الامتصاص عند أعداد موجية معينة، بينما R^2 هو معامل التحديد.

طُبقت طريقة ATR–FTIR المُعتمدة لتحليل عدة عينات من الدقيق الأبيض التجاري التي جُمعت من السوق الفلسطيني. وقد أظهرت النتائج خطية وحساسية وقابلية تكرار ممتازة لجميع المواد المضافة المدروسة. علاوة على ذلك، كشفت الدراسة عن وجود تراكيز متفاوتة من برومات البوتاسيوم،الألوكسان، ويودات البوتاسيوم في بعض عينات الدقيق التجارية، مما يشير إلى مخاوف محتملة تتعلق بالصحة العامة. توفر الطريقة المقترحة بديلاً سريعًا، وبأقل قدر من تحضير العينات، وفعالًا من حيث التكلفة، وغير مُتلف للعينات، مقارنةً بالتقنيات التحليلية التقليدية – مثل كروماتوغرافيا السائل عالي الأداء (HPLC) والمعايرة – ويمكن تطبيقها بفعالية في المراقبة الروتينية والتقييم الكمي لمضافات الدقيق لدعم سلامة الغذاء، ومراقبة الجودة.

الكلمات المفتاحية: المواد المضافة،الطحين، برومات البوتاسيوم، الألوكسان، يودات البوتاسيوم، ATR–FTIR، منحنيات المعايرة، الطرق التحليلية.