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THE OPTIMAL NUTRITIONAL COMPOSITION IN THE BREAD MAKING BASED ON A LOCALLY SUSTAINABLE WHEAT SUBSTITUTES

A study thesis submitted as partial fulfillment requirement of the Bachelor degree in Clinical Nutrition and Dietetics

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Dedication:

Who those who once told us you
will be what you want and here we are
about to reach your words…
To the loving hand that has stuck
to our hands to reach the impossible,
for every catalyzing look that made us
what we are now…
To our mothers and fathers and to
all who were next to us

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Abstract

Bread has been considered as the staple food of choice in Yemen, bread is traditionally produce it from wheat (*Triticum aestivum*) and due to high demand and lower domestic production; about 95% of needed wheat is imported from some countries like Australia, Russia, Ukraine, USA, India with seven hundred million dollars annually and because of the growing costs of imported wheat and inability to sustain the national wheat imports for making wheat based foods, makes is imperative to substitute wheat with other locally crops.

In this study, nine bread formulations were prepared by total substitution of some locally crops like wheat flour with quinoa flour, red lentils, pumpkin, barley, sesame, red corn, yellow corn, and millet flour for evaluation of composite bread making with compared to wheat flour (control).

Chemical and rheological examinations of all samples showed that sample No. 4 (M4) is the best sample in terms of nutrient content. The study also showed that the microorganisms in all samples were within the acceptable limits of the standards of the World Health Organization and the Food and Agriculture Organization, they are absolutely fit for consumption immediately after cooking. The quality of all prepared composition (formulations) was assessed for acceptability by trained panel members using a five-point hedonic scale, although several formulations were sensory acceptable. The scores were largely similar, but the M4 formulation was highly acceptable to the committee members and scored significantly higher scores ($P < 0.05$) than the other wheat alternative samples, where their average score ranged between 3.50 to 3.94 in terms of taste, flavor, texture, appearance and overall accept.

In general: this study showed that these locally crops are suitable for developing bread of good technological quality and improved nutritional profile, adding value to these underused ancestral flours.

Keywords: Yemen, composite flour, wheat total substitution (locally crops), rheological and microbiology.

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1. Introduction

Bread is known as one of the most ancient foods and widely consumed in all its various forms by humanity. Wheat grains contain unique gluten proteins that impart viscoelastic properties dough required for leavened bread making[1]. In the Western world, refined wheat flour has been the standard raw material for bread production[2]. The consumption of refined wheat bread has been increasing rapidly in the developing countries due to urbanization and industrialization[3], and is associated with the burden of non-communicable diseases[4]. Meanwhile, ~3 billion people, most of which are in Asia and Africa, could not afford a healthy diet in the pre-pandemic period[5]. A primary driver of this increasingly dire situation is the double burden of climate shocks and violent conflict in areas that are already food insecure[6].

In Yemen, bread is traditionally produced from wheat "*Triticum aestivum*" flour. Due to high demand, about 95% of needed wheat is imported with seven hundred million dollars annually. Therefore, inability to sustain the national wheat imports for making wheat based foods, makes is imperative that some substitutes for wheat must be incorporated in the bread preparation. Alternative non wheat cereals that has capacity to substitute wheat in bread flour like Quinoa, Red lentils, Pumpkin, Barley, Sesame, Teff, Red corn and Yellow corn flours[1].

On the other hand, in developed countries, consumers are increasingly aware of the health and environmental benefits of bread products produced partially using non-wheat ingredients, which are thought to be low in glycemic index (GI; a value used to measure how much specific foods increase blood sugar (glucose) levels), rich in protein, dietary fiber and various bioactive compounds[2].

The concept of reducing wheat importation by replacing part of it with indigenous crops in food production in developing countries dates back to the 1960s, which was envisioned to increase food security in vulnerable regions. In the context of bread making, the bread produced by using a combination of wheat and wheat flour substitutes has been described as composite bread. Despite the growing interest in composite bread in recent years, the development of composite bread has been primarily limited to home baking and its associated research is relatively scant (Fig. 4). Among other factors, low consumer acceptability and unfamiliarity with the benefits of composite bread represent major obstacles[3,4].

Recently, the processing strategies for improving the quality of composite bread have gained increasing interest, and sustainable bread production becomes imperative in the post-crisis era. Therefore, in this study, we prepared of wheat flour substitutes to sustainable bread making from a combination of local crops were teff, quinoa, lentils, barley, sesame, red corn, yellow corn, pumpkin and millet as well as other additives using household- and industrial-level approaches to improve their techno-functionality, sensory characteristics, and nutritional values.

This research will be study of replacing wheat flour with alternatives from sustainable local crops (Quinoa, Red lentils, Pumpkin, Barley, Sesame, Teff, Red corn and Yellow corn flours) and to improve nutrient value and reduce the energy consumption, the cost and adding value to underused ancestral crops.

2. Literature Review

2.1 General context:

Yemen is located on the southwestern edge of the Arabian Peninsula, with a total area of 527,970 square kilometers. It is bordered by the Kingdom of Saudi Arabia to the north, the Sultanate of Oman to the east, the Arabian Sea and the Gulf of Aden to the south, and the Red Sea to the west, as shown in (Map 1)[1].

Figure 1 The map of Republic of Yemen

2.2 Population of Yemen

According to the statistics issued by the World Bank (WB) for the year 2021, the population of the Republic of Yemen amounted to approximately 30,042,375 people, or 0.38% of the total world population. The population growth rate in 2020 was 3.7%, as shown in (Figure 2)[5-8].

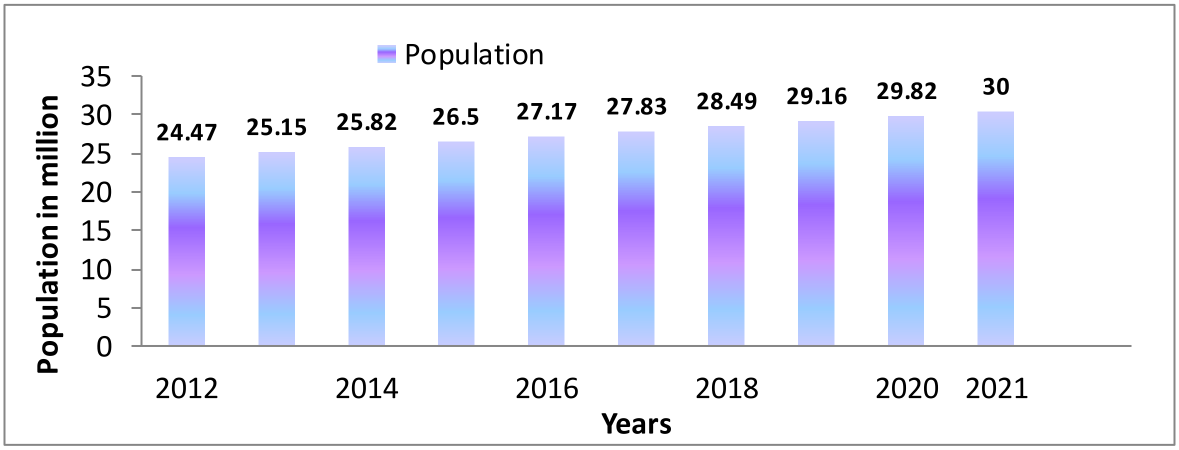


Figure 2 Population of Yemen

2.3 Food Security

Food security is one of the main challenges facing the Republic of Yemen, and increasing the production of agricultural crops to meet the needs of local consumption of food commodities is one of the main ingredients for achieving food sufficiency[8]. The agricultural sector in Yemen is considered one of the most important productive sectors, but rather the only sector responsible for producing quantities of food that a person cannot do without. The average contribution of the agricultural sector to the national income (16.5%) of the Republic of Yemen, and the agricultural sector comes first in terms of labor absorption, as the percentage of the agricultural labor force reaches 54% of the total labor force in the country[8]. The arable area in the Republic of Yemen is (1,609,484) hectares, while the cultivated area represents about (1,452,438) hectares (90%)[9]. The following table shows the volume of agricultural crop production in the Republic of Yemen during the period 2016-2021[8].

Table 1 the quantity of production (tons) of agricultural crops in Yemen 2016-2021

Cereal	2016	2017	2018	2019	2020	2021
Grains	357,068	358,355	344,648	456,714	789,527	879,342
Wheat	95,917	95,651	92,210	100,332	127,171	138,027
Sorghum	162,277	164,241	155,722	230,766	474,676	522,779
Millet	44,587	44,275	43,390	50,393	64,786	82,526
Maize	36,892	36,887	36,438	48,290	86,159	94,375
Barley	17,395	72,630	62,486	93,139	36,735	41,635
Qat	186,285	185,621	186,167	237,299	241,309	291,498

Source: Annual Agricultural Statistics Books, 2020, 2021 Link, Link

In addition to, the figure 3 shows Quantity of import (tons) of wheat in Yemen 2014-2021[9].

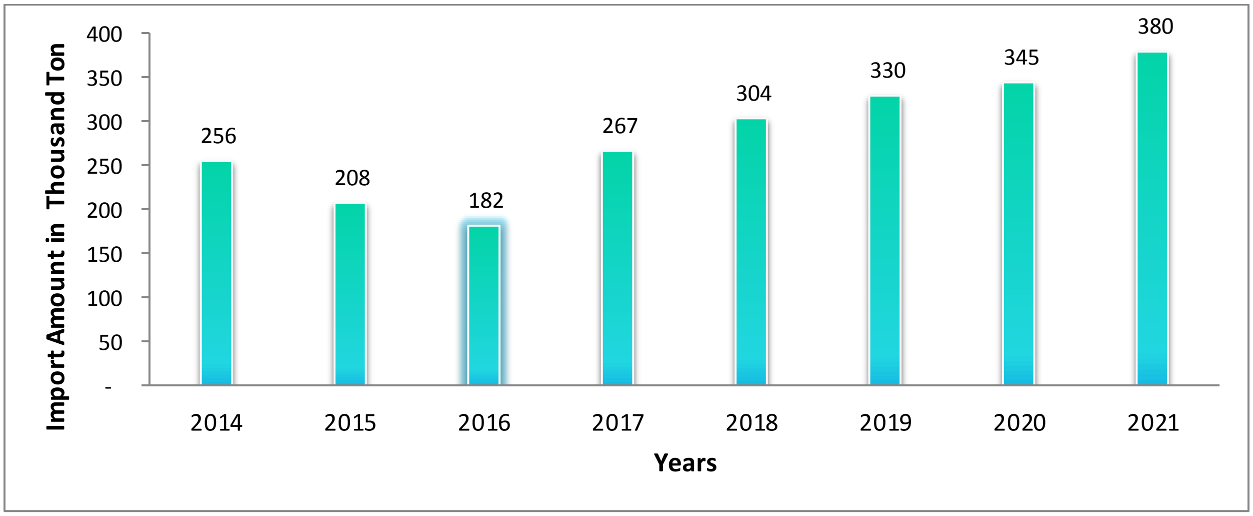


Figure 3 Quantity of import (Thousand Tons) of wheat in the Republic of Yemen 2014-2021

2.4 Wheat flour substitutes in sustainable bread making

The consumption of refined wheat bread has been increasing rapidly in the developing countries due to urbanization and industrialization[7], and is associated with the burden of non-communicable diseases[7]. In Yemen, bread is consumed at higher ratios than other countries. It is reported that the amount of daily consumption ranged from 250-320 g per capita according to the imported quality of wheat[6]. Meanwhile, ~30 million people in Yemen could not afford a healthy diet due to climate shocks and violent conflict since 2015[6]-[8], in addition to negative environmental and health consequences[8].

Therefore it is necessary to provide sustainable, sufficient, appropriate and accessible resilient food system to all by partially or completely replacing wheat flour with various types of plant ingredients for bread making, also known as composite bread[9], which are thought to be low in glycemic index (GI), rich in protein, dietary fiber and various bioactive compounds[9-10]. They are also supposedly lower in the carbon and water footprint compared to refined wheat bread, contributing to environmental sustainability[11,12]. The (Figure 4) showed number of publications per year in the FAO database related to composite bread over the past 20 years (2014–2021)[13].

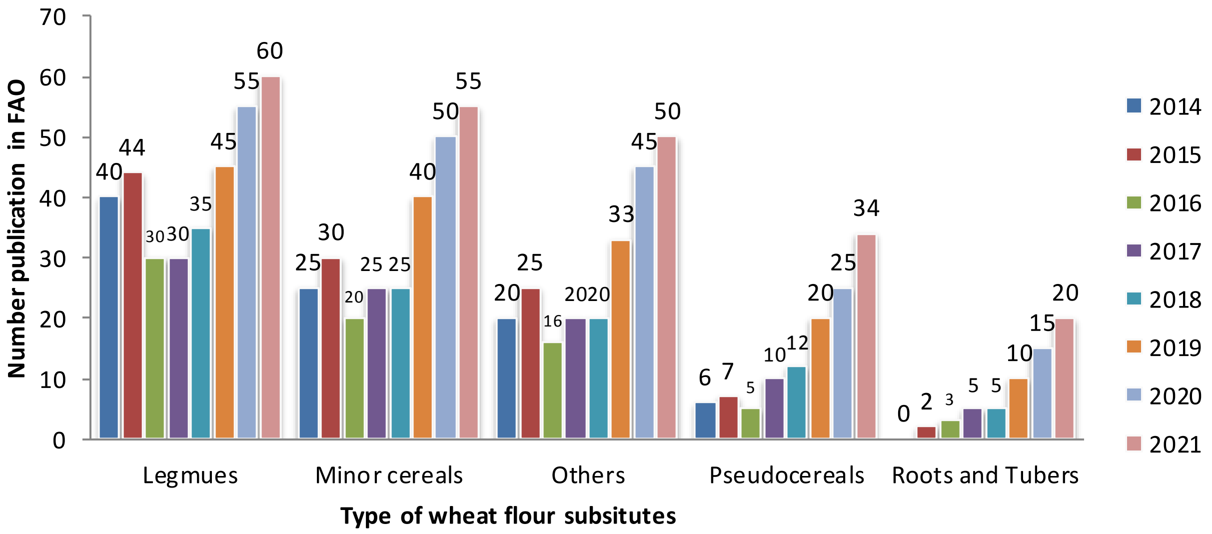


Figure 4 Number of publications per year in the FAO database related to composite bread over the past 20 years (2014–2021).

2.5 The negative consequences of dependence on wheat flours

In terms of production, wheat's 752 million tons globally (Mt) over the 5-year period from 2015 to 2020 is slightly less than rice (768 million tons[12]. China is the leading wheat producer, accounting for 17.6% of the world total wheat production in 2020, whereas the other top producers e.g., India, Russian Federation, the United States of America, Canada, and France account for 40.5%5. It is estimated that wheat production should increase by 87 Mt to 840 Mt by 2030 to meet future food demands[19]. The drastic increase in wheat cultivation has intensified the need for sustainable food production. On the other hand, wheat consumption is expected to increase by 12% by 2030, where more than two-thirds are used for food[9]. The adoption of western lifestyle and diet due to urbanization an industrialization in developing countries is the major driving force for increasing wheat demand[13] (Fig. 5).

The increase in wheat consumption is especially concentrated in Africa and the middle East/Western Asia, most of which are beyond the regions of wheat production and heavily rely on wheat imports that are susceptible to systemic disruptions[13] (Fig. 5). The COVID-19 pandemic and the recent Russian-Ukraine armed conflict, which both have longlasting ramifications in wheat production and supply chain disruptions, have added more pressure on food system resilience with negative consequences for food security in these vulnerable regions in the years to come. The current share of global wheat importation by Africa and the Middle East/Western Asia is ca. 45% and is predicted to rise due to increased adverse weather events (e.g., rising temperatures and declining rainfall) accentuated by climate change. Climate change causes volatility in crop yields and fluctuations in wheat prices, leading to uncertainty about future wheat availability in the vulnerable regions[12]. Moreover, wheat, rice, and maize are responsible for up to 60% of nutrient runoff globally[13]. It has been estimated that over 50% of the environmental impact of producing an 800-g loaf of wheat bread arises directly from wheat cultivation, with the use of ammonium nitrate fertilizer alone accounting for around 40%[24]. This negative environmental impact perpetuates a vicious cycle, increasing the fragility of the global food system. Low- and middle-income countries now experience the highest prevalence and mortality rates of cardiovascular disease[15].

The increased use of refined wheat flour in current bread making practices has been associated with a higher risk of mortality and major cardiovascular disease events[25]. On the other hand, the consumption of bread made of whole-grain cereals or enriched with bioactive compounds is generally recognized as health promoting[16], and has been explored as an approach to improve cardio metabolic profile[14]. Taken together, sustainable bread production becomes imperative in the challenging time. This will require a fundamental transformation of current practices that rely predominantly on wheat grains, preferentially in ways that prioritize the needs of vulnerable regions as the impacts of food insecurity are highest in these regions[16].

2.6 Substitution of wheat flour as a solution to sustainable bread production

Unlike refined wheat flour, many non-wheat cereals and legumes like Quinoa, Red lentils, Pumpkin, Barley, Sesame, Teff, Red corn, Yellow corn and Millet possess dense nutritional composition and a range of health-promoting bioactive compounds and dietary fibers with diverse structures and it contributes in bread making and local economic development[17,18].

For instance, wheat contains lower concentrations of β-glucan that differs from Teff, Quinoa and barley β-glucans in molecular structure[19]. Teff, Quinoa and barley β-glucans are relatively more soluble and shown to maintain gut health by various mechanisms, including modulation of the gut microbiota[18].

Hence, substitution of Quinoa, Red lentils, Pumpkin, Barley, Sesame, Teff, Red corn, Yellow corn and Millet in wheat-based foods diversifies dietary fiber sources, which is conductive to gut and metabolic health[17].

Minor cereals the above-mentioned in food production can be thus leveraged to correct our fiber-impoverished modern diet[13]. Therefore, improved food security and food system resilience, human health, and reduced environmental cost can be integrated into a common framework of sustainable bread production through the substitution of wheat flour.

Diversification of plant-based food sources is necessary to improve the sustainability in global food systems. In addition to reduced environmental impact, utilization of indigenous grain crops in industrial processes contributes to local economic development[13].

The shorter food supply chains provide easier access to healthy and affordable food in crisis situations, promoting food system resilience[13].

The following figure shows the amount of global production) million tons) of barley, buckwheat, millet, oats, quinoa, and sorghum as well as the amount of consumption[13].

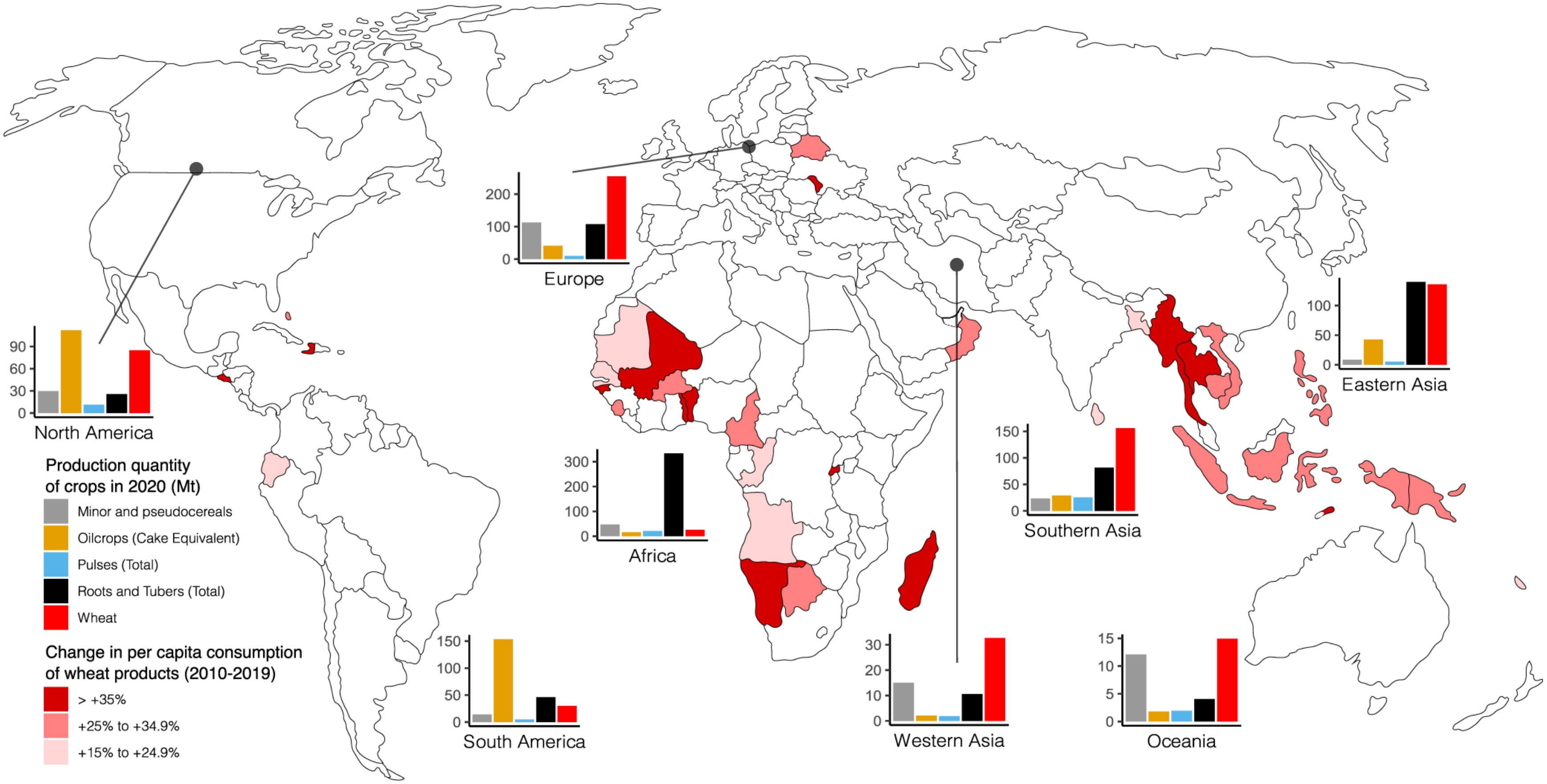


Figure 5 grouped according to FAO's categories, whereas minor and pseudo cereals include barley, buckwheat, millet, oats, quinoa, and sorghum.

The unit of production quantity is million tons (Mt). The production quantity of individual countries is aggregated to the indicated regional level according to FAO's categories. The consumption data is expressed as relative change. Data from FAO5: https://www.fao.org/faostat/en/#data, accessed 30/04/2022).

2.7 Sources of wheat flour substitutes

2.7.1 Legumes

Plant-based proteins are considered more environmentally friendly than animal proteins due to their lower carbon footprint, land use, and water use[18]. From a nutritional point of view, incorporating plant protein (Red lentils and Pumpkin) ingredients in wheat bread contributes to a higher protein intake with a better amino acid profile[19]. For instance, symptoms of tryptophan deficiency, including reduced growth, impaired bone development, and neurological abnormalities, often occur in parts of sub Saharan Africa where maize, known to be deficient in tryptophan, is the staple food[39]. Some legumes like Red lentils and Pumpkin a are natural sources of tryptophan[20]. Legumes are well known for their nitrogen fixing ability that reduces the emission of greenhouse gases in agro-ecosystems[21]. They also have low carbon and water footprints; food legumes occupy a minimal part of arable land[22]. The consumption of legumes has been suggested to provide health benefits via their antioxidant activity, blood pressure lowering, hypoglycemic, hypocholesterolemic, antiatherogenic, anticarcinogenic, and prebiotic properties[43]. Legumes are rich in dietary fiber (8–28 g/100 g) and protein (21–37 g/100 g)[22].

Proteins sourced from legumes are categorized as incomplete proteins, since they tend to be low in the essential amino acids such as methionine and cysteine. Complete proteins from legume based foods can be complemented by other crops such as cereals[17,22], making them attractive ingredients for composite bread.

Composite breads using various types of legume flour are among the most studied wheat flour substitutes (Fig. 4). They can be integrated in bread formulations as either flours or protein isolates (protein content >90%) or concentrates (protein content 60–75%). Findings from previous research have demonstrated that partial substitution of wheat flour with legumes in bread making confers the bakery products with a better amino acid profile, specifically by complementing the deficiencies of lysine and threonine in wheat and inadequate sulfur amino acids in legumes, fulfilling the nutrition[17]-[19].

2.7.2 Minor cereals

Minor cereals (e.g., sorghum, millets, barley, Teff, oats, quinoa, buckwheat, and amaranth play an important role in composite flour making. These crops have remained largely neglected in commercial food production due to the lack of processing technologies, and therefore the consumption is restricted mainly to their growing regions. On the other hand, findings from recent studies support the prebiotic properties and beneficial metabolic effects of millets[23], barley and oats[55], buckwheat[55] and quinoa[24], incentivizing functional food minor cereals.

Sorghum (Sorghum bicolor L), Teff and millets are important food items in South Asia and sub-Saharan African countries, accounting for a large part of total caloric intake[17]. Sorghum ranks fifth in the global cereal crop production followed by pearl millet (Pennisetum) and teff (Eragrostis).

Sorghum, Teff and millets are commonly consumed as whole grains in traditional cuisines, such as roti (unleavened breads or pancake) and porridge. They are nutritionally analogous to conventional cereals (on average 65% carbohydrates, 10% proteins, 3.5% fat, and 8% dietary fiber) and serve as an excellent source of micronutrients (vitamins, e.g., B vitamins and vitamin E, and minerals, e.g., magnesium, phosphorous and iron), and phytochemicals (phenolic acids, tannins and flavonoids)[26].

The consumption of sorghum, teff and millets has been linked to a multitude of health benefits, such as weight control[25], lowering serum cholesterol and triglycerides levels[27], reduction in starch digestibility and improvement of blood glucose control[24], and mitigation of gastrointestinal disorders including the risk of colon cancer[28].

Barley (Hordeum vulgare L.) is the fourth most important cereal, and its largest producer is the European Union, followed by Russia, Ukraine, and Australia. Barley is used predominately as animal feed (ca. 70%) and to a less extent as a brewing raw material (ca. 21%), and only 6% is consumed by humans[29]. In recent years, the consumption of barley and oat-based products (e.g., breakfast cereals, porridge, and unleavened bread) has substantially increased due to consumer awareness regarding their nutritional values (e.g., β-glucans and antioxidant compounds) and health claims. Barley and oat grains contain high levels of β-glucan, 2.5–11.3% and 2.2–7.8%, respectively[29]. The consumption of ≥3 g barley or oat β-glucan per day (i.e., 0.75 g/ serving) has been acknowledged by the United States Food and Drug Administration (FDA) and the European Food and Safety Authority (EFSA) to have health claims, such as lowering postprandial glycemic and insulin responses, lowering serum cholesterol and lipid levels, immune stimulant activity, reduced risk of colon cancer, preventing type 2 diabetes, and improving gastrointestinal function (via increasing the apparent viscosity in the upper digestive tract)[30]. β-glucans appear to remain intact following the baking process, but high levels of oat or barley flour needs to be added to wheat dough to meet the health claims (ca. more than 50%)[28].

In a recent study, oat fiber (70% of β-glucan) was used in bread making by substituting 10–14% of wheat flour with it, resulting in a bread product with 3.4–4.6 g β-glucan/100 g serving60. Furthermore, the incorporation of 60% barley flour and 5–20% oat bran enriched the dietary fiber content, total phenolic content, and enhanced the antioxidant activity of the final breads[30].

Quinoa and amaranth are ancient crops mainly grown in South America, such as Peru and Bolivia. Buckwheat is mainly produced and consumed in Russia and China, followed by Ukraine and the United States. These crops are climate resilient with little water demand and good tolerance against heat, drought, and soil salinity compared to cereals[31]. Furthermore, pseudocereals (Quinoa and amaranth) have a higher nutritional value than wheat and rice.

Quinoa, amaranth, and buckwheat are rich in protein (on average 14%) with a well-balanced amino acid profile and are good sources of dietary fiber (14.6%), unsaturated fatty acids (4.7%), vitamins (ascorbic acid, tocopherol, carotenoids, folate, riboflavin, and thiamine), minerals, and bioactivecomponents (e.g., polyphenols, saponins, betalains, phytosterols, and bioactive peptides) [30].

2.8 Challenges in bread making using wheat flour substitutes

2.8.1 Key features in wheat bread making

The textural and sensory qualities of wheat bread are often considered as a benchmark for composite bread. Wheat bread making is a multistage dynamic process with several essential features, including mixing of the ingredients, development of a gluten network from kneading, incorporation of air bubbles, fermentation in which CO_2 produced by yeast is entrapped in air bubbles, baking, crust formation, surface browning reaction, and formation of the cellular structure in final bread[32]. Upon mixing, the gluten proteins (i.e., gliadins and glutenins) are hydrated and a three-dimensional gluten network (disulfide (SS) bonds) is formed with air cells being trapped in this matrix. During yeast fermentation, the produced CO_2 dissolves in the aqueous phase of the dough until saturated, and then diffuses to the existing cell nuclei while some CO_2 escapes. The retention of gas bubbles is essential for the liquid foam structure of the dough. The gluten network, which creates the viscoelastic properties of bread dough, plays a crucial role in gas holding and dough development[33].

2.8.2 Techno-functional challenges in composite bread making

Flours of other crops may not be conventionally processed in bread making due to significantly different properties of their proteins compared to wheat gluten. Using wheat flour substitutes in bread making at high levels usually produces final products of unacceptable quality. In general, the substitution levels above 10% lead to a decrease in bread specific volume and an increase in crumb hardness.

The effects of adding non-wheat flours on the rheological properties of dough, e.g., farinograph water absorption, starch pasting profiles, dough extensibility, and viscoelasticity (elastic modulus and viscous modulus), and have been extensively investigated. The addition of fiber-rich ingredients derived from legumes, barley, oats, and BSG often results in increased water absorption, whereas the opposite effect occurs with the addition of starchy ingredients, such as millets and root flours. Moreover, incorporating wheat flour substitutes at high levels leads to longer dough development time, higher starch gelatinization temperature, lower dough stability and extensibility, decreased gluten strength and elasticity, and increased dough stickiness[34,35].

These negative impacts are related to a weakened gluten network, where **(1)** gluten protein hydration is reduced due to the competition of water between gluten proteins and fibers or non-wheat proteins; **(2)** the formation of the gluten network is disrupted due to the different functional properties of non-wheat proteins; (3) the gluten secondary structure is altered[36].

2.9 Sensory challenges in composite bread making

Consumers crave foods that satisfy the sensory qualities they enjoy, such as mouth feel, taste and aroma. Flavor is the combination of aroma, taste and chemisthesis. Taste is due to the non-volatile compounds present in food described as sweet, salty, bitter, sour, and umami. Aroma is related to volatile compounds. The off-flavors present in wheat flour substitutes, such as beany flavor, bitter taste, and aftertaste represent a major hindrance toward consumer acceptability130. The enrichment of wheat bread with legume-based ingredients at higher levels often leads to a beany flavor. For example, the inclusion of soy flour above 10% generated a strong beany flavor and an aftertaste, resulting in lower flavor ratings and taste acceptance than the wheat control[62]. The incorporation of 10% lupin protein isolate generated beany, earthy, and malty notes in the bread[62].

Legume seeds contain 2–20% lipids with a high level of unsaturated fatty acids: oleic (4–38%), linoleic (28–55%) and linolenic (3–37%) acids[34]. The oxidation of unsaturated fatty acids plays a crucial role in the development of off-flavor compounds in legume-based products[29]. This oxidation can be enzymatic or non-enzymatic (auto-oxidation and photo-oxidation).

Legumes, e.g., soy, faba bean, and pea, are rich sources of lipid degrading enzymes, such as lipoxygenase and lipase. Lipase catalyzes the hydrolysis of triglycerides to free fatty acids. Lipoxygenase catalyzes the degradation of polyunsaturated fatty acids to produce hydroperoxides, which are subsequently degraded in enzymatic or chemical reactions forming volatile and non-volatile compounds responsible for off-flavors[35]. Hexanal, 3-cis-hexenal, 2 pentylfuran, (E,E)-2,4-decadienal, and ethyl vinyl ketone are identified as major lipoxygenase derived contributors to beany and green notes[35]. These off-flavor compounds are detected at low threshold values and thus a small quantity of fatty acids is enough to develop a strong beany off-flavor.

Bread enriched with wholegrain or fiber-rich ingredients has the organoleptic characteristics often described as bitter, astringent, and rancid, which is related to the presence of free fatty acids, saponins, alkaloids, isoflavones, phenolic acids, tannins, small peptides, or amino acids, or combinations thereof[39]. Sorghum contains a significant amount of polyphenols and condensed tannins contributing to bitterness and astringency[36]. The addition of 50% wholegrain sorghum flour in wheat bread led to higher intensities of bitter taste and aftertaste compared to 100% wheat bread[37].

Oat flour, having high lipid content (4–8%), is susceptible to lipid oxidation where the produced long-chain hydroxyl fatty acids confer a bitter taste, and its volatile compounds impart a rancid off-flavor[36].

The incorporation of 5–15% barley protein isolate induced an intense bitter taste of wheat bread[39]. BSG has a typical malt flavor developed during the mashing process and a bitter taste140. Bread supplemented with BSG at above 10% had more intense bitterness and acidic flavor[37]. Adding BSG or oat bran to wheat flour at levels higher than 10% reduced the sensory scores for odor, taste and overall acceptability[40]. Legumes, such as faba bean, lentil, and soy, contain a considerable amount of saponins (saponin βg and saponin Bb), which are perceived as bitter, astringent, and metallic[35]. Lupin (Lupinus albus L.) is rich in alkaloids with a strong bitter taste and needs to be debittered prior to bread making[41]. For this reason, the Australian sweet lupin (Lupinus angustifolius), which contains very low levels of bitter alkaloids, is a preferred option in bread fortification[33]. Furthermore, the offtaste compounds and precursors in plant raw materials are often retained during the protein isolation process due to their interactions with proteins (e.g., bitter-tasting kaempferol derivatives in rapeseed protein isolates), causing a negative sensory perception[42].

2.10 Nutritional challenges in composite bread making

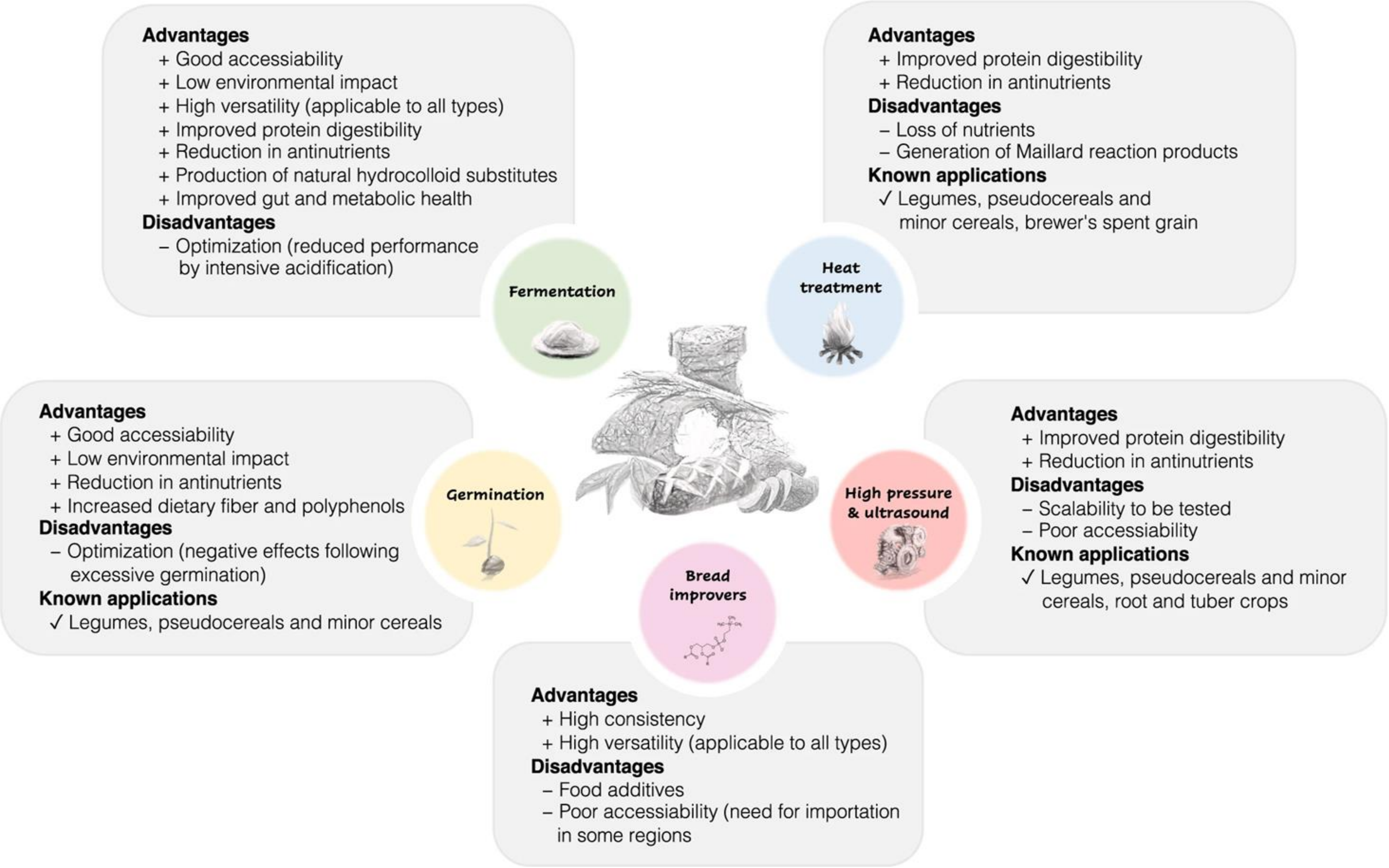
Plant-based ingredients contain certain phytochemicals naturally produced as secondary metabolites by plants[43]. As part of the plants' defense mechanism against being eaten, these bioactive compounds almost always confer off-tastes in addition to disrupting the bioavailability and utilization of nutrients and minerals in animals[43], and hence are dubbed as "antinutrients". Antinutrients are sometimes referred to as non-nutrients since some studies claim that they possess health promoting effects when in the appropriate quantity and under the right conditions[40]. Notwithstanding their ambivalent properties that require further research, elimination or reduction of antinutritional factors is the target in most food production. Oilcakes contain antinutrients such as phytic acid, glucosinolates, sinapine, cyanogenic glycosides, trypsin inhibitors, and tannins[44]. Sinapine (bitter taste) is the major phenolic constituent in rapeseed meals, which forms complexes with proteins via oxidation and decreases digestibility[45]. Glucosinolates (bitter taste) have been shown to have goitrogenic and anti-thyroid effects in both humans and animals[45] . Cyanogenic glycosides (bitter taste), the principal antinutrient in flaxseed meals, can produce toxic hydrogen cyanide following the breakdown in the gastrointestinal tract[46]. Linatine is also found in flaxseed meals that can cause pyridoxine (vitamin B6) deficiency[46]. Phytic acid, present in most oilcakes, can bind to minerals, proteins, and amino acids. This reduces their bioavailability and inhibits the activity of α-amylase, leading to decreased starch digestibility. Tannins (bitter and astringent) can precipitate proteins and reduce the absorption of minerals, particularly iron. Trypsin inhibitors are known to reduce the digestibility of proteins[45].

Legumes also contain high concentrations of antinutrients such as phytic acid, lectins, vicine and convicine, enzyme inhibitors (trypsin, chymotrypsin, and α- amylase inhibitors), condensed tannins, saponins, and flatulent causing oligosaccharides[47]. Lectins are carbohydrate-binding proteins widely distributed in leguminous crops. Legume lectins negatively affect the functions of human digestion system and nutrient absorption due to their binding to the intestinal epithelial cells[47].

Vicine and convicine cause a severe haemolytic anemia, known as favism, in susceptible individuals with the deficiency in the glucose-6-phosphate dehydrogenase enzyme[48]. The indigestible raffinose family oligosaccharides (RFOs), such as raffinose, stachyose, and verbascose, are abundant in legumes. While several studies suggest their prebiotic potential, the high intake of RFOs causes abdominal discomfort and diarrhea in some people via gas production derived from increased colonic fermentation[49]. Pseudocereals contain saponins, phytic acid, tannins, and protease inhibitors[49]. Saponins are particularly abundant in quinoa, which cause hemolysis by reacting with the sterols of erythrocyte membrane and interfere with the absorption of lipids, cholesterol, bile acids and fat-soluble vitamins[50]. Phytic acid and tannins are major anti-nutritional components present in sorghum, millets, and BSG[50].

2.11 Improve physicochemical and sensory attributes of composite bread

The altered textural, sensory and nutritional and presence of food additives in wheat flour substitutes represent a major limitation in their utilization and eventual consumer acceptability. Several processing strategies have been applied to produce composite bread with technological and sensory profiles comparable to refined wheat bread. Main advantages and drawbacks of the different processing strategies, in addition to textural and sensory improvements, are summarized in Fig. 6. Few strategies are universally effective for all types of wheat flour substitutes, and therefore optimization of conditions for specific ingredients or combinations of strategies are often needed to achieve desirable outcomes.



Fermentation

Advantages
- + Good accessibility
- + Low environmental impact
- + High versatility (applicable to all types)
- + Improved protein digestibility
- + Reduction in antinutrients
- + Production of natural hydrocolloid substitutes
- + Improved gut and metabolic health

Disadvantages
- – Optimization (reduced performance by intensive acidification)

Heat treatment

Advantages
- + Improved protein digestibility
- + Reduction in antinutrients

Disadvantages
- – Loss of nutrients
- – Generation of Maillard reaction products

Known applications
- ✓ Legumes, pseudocereals and minor cereals, brewer's spent grain

Germination

Advantages
- + Good accessibility
- + Low environmental impact
- + Reduction in antinutrients
- + Increased dietary fiber and polyphenols

Disadvantages
- – Optimization (negative effects following excessive germination)

Known applications
- ✓ Legumes, pseudocereals and minor cereals

High pressure & ultrasound

Advantages
- + Improved protein digestibility
- + Reduction in antinutrients

Disadvantages
- – Scalability to be tested
- – Poor accessibility

Known applications
- ✓ Legumes, pseudocereals and minor cereals, root and tuber crops

Bread improvers

Advantages
- + High consistency
- + High versatility (applicable to all types)

Disadvantages
- – Food additives
- – Poor accessibility (need for importation in some regions

Figure 6 Improving sensory and nutritional quality of composite bread

2.12 Future prospects

In this review, we have discussed the benefits and challenges associated with composite bread making using various types of locally wheat flour substitutes, the demand and research of which will likely increase exponentially in the near future. Going forward, interdisciplinary approaches addressing the current knowledge gaps in the environmental, nutritional, health and technological dimensions are required. The synergism between sustainability diet, human nutrition, microbiomics and food science is necessary to scale up research results for large-scale positive impact[51].

In the environmental dimension, life cycle assessment (LCA) studies on the ingredient-to-bread chain are warranted to understand the environmental impact of composite bread made of different wheat flour substitutes. A comparative approach simultaneously evaluating the nutrient density per unit environmental impact per serving should be devised to identify candidates that are both nutritionally dense and environmentally sustainable. Chaudhary *et al.* developed the nutrition carbon footprint score (NCFS) as an indicator of product-level nutrient density per unit environmental impact by combing nutritional profiling systems with LCA analysis[43].

Such nutritional profiling systems can be flexibly adapted to the nutritional needs of specific regions. For instance, in the Chaudhary study, the nutrient balance concept (NBC) was used, in which an aggregated measure is calculated based on nutritional quality, i.e., whether a nutrient is considered to have a positive or negative effect on the nutritional profile of a given food[51]. Using this approach, the authors proved that the food products made of yellow pea-wheat composite flour had higher nutrient density per unit environmental impact compared to their refined wheat counterparts[52].

The LCA analysis incorporating nutrient density is particularly important for comprehensively understanding the benefits of food waste valorization, as processing food waste into edible ingredients may increase its environmental impact in some scenarios[37]. Future studies of this kind are essential as basis for policy making involving different stakeholders to improve the knowledge of what to eat and develop relevant processing technologies for sustainable food production that promotes wellness, especially in vulnerable regions. Foods would generally improve environmental sustainability[37]. Moreover, nutritional and health benefits are instrumental in promoting the acceptance of sustainable composite bread.

Consumers from developed countries are increasingly interested in selecting "gut-friendly" bread on the market[52]. These underscore the importance of conventional food trials to evaluate the effect of food products, e.g., newly developed composite bread, on consumer health[29]. Traditionally, nutritional studies have taken a reductionist approach, focusing on the constituent nutrients of a food; food science and technology has been based on a whole-food approach, placing a greater emphasis on food morphometry and physico-chemical properties. The multiplicity of interactions between nutrients in whole foods often change their nutritional performance and health potential[52].

Therefore, it has been proposed that future research needs to unite the two approaches, using “food” as a fundamental unit to investigate its effects on multiple surrogate endpoints[22], including the gut microbiota. Albeit with inter-individual variation, a person’s gut microbiota can be used to gauge their health status[42], Therefore, it is necessary to include microbiomics in all conventional food trials, as it broadly reflects health consequences of food products and their processing technologies. The latter has not been rigorously evaluated[20], while the relationship between processing and the food matrix, and the resulting implications in digestion, nutrition and health are a subject of recent interest. For instance, multiple studies have shown that processing techniques in bread making have a significant impact on post-prandial metabolic responses[52]. Currently, studies on the effectiveness of different processing techniques in reducing antinutritional compounds and their health implications in composite bread are lacking.

In terms of processing technologies, strategies to modify processing variables during bread making, such as lactic acid fermentation, remain underutilized for bread preparation from non-wheat grains[33].

We believe that fermentation with in situ produced dextran is one of the most versatile and accessible approaches for improving textural and sensory properties of composite bread. Thus, future studies would benefit from a mixand- match approach, where the investigations focus on what optimal combination between fermentation and other methods is required to increase the proportion of wheat flour substitutes with minimum impact on the nutritional and sensory attributes of the bread. Fermentation of plant-based ingredients, either by autochthonous microbes present in the raw material or with selected starters, has been traditionally used in preparing foods and consumed in many indigenous communities in Africa, Asia, Europe, and the Americas[27].

The cultural resurgence of sourdough provides an excellent example, showcasing that these traditional fermented foods represent a treasure trove of resources that could be harnessed to improve health and food quality. A recent study profiling the microbiotas in a large collection of sourdough starters found that acetic acid bacteria, a mostly overlooked group of sourdough microbes, are responsible for the variation in dough rise rates and aromas[51]. It is therefore tempting to characterize the microbial communities in different fermented foods and identify specific microbes responsible for their unique flavors and aromas. The fermentation process can be subsequently adjusted to produce bread products with organoleptic properties similar to the fermented foods with which locals are familiar. The familiarity will likely increase local consumers preference even if the bread products are subpar to refined wheat bread in some aspects[52].

3. Methods and Materials

3.1 Samples collection

Quinoa, Red lentils, Pumpkin, Barley, Sesame, Red cornو Yellow corn, Millet and wheat (control) as well as ginger and anise were purchased from a local market in Sana’a, Yemen, while the Teff seeds we got from the Agricultural Research Authority in Dhamar Governorate, Yemen. All of samples collected on August 2022 (Figure 7).

Figure 7 local crops and Flavors used in study

3.2 Study area

Preparation of composite flour mixes; bread making and evaluation of sensory were carried out in Department of Therapeutic Nutrition and dietetics, Collage of Medicine and Health Science, University of Science and Technology, Sana’a, Yemen. While Chemical analysis and rheological properties were carried out in the Al Snabel milling plant located at Aden city (400 km from Sana’a As for the microbial analyzes of the composite flour mixtures, they were carried out in the Collage of Science at Sana'a University, Sana’a, Yemen. The study began in September 2022 and ended in January 2023.

3.3 Composite flour preparation

Mixing is most important step in preparing composite flours or bread, during which all the ingredients are mixed and distribute as indicated in the (Table 2)[53].

Table 2 Formation of composite flours mixers

Mixers (g/100 g)	**First mix (M1)**	**Second mix (M2)**	**Third mix (M3)**	**Fourth mix (M4)**	**Fifth mix (M5)**	**FSixth mix (M6)**	**Seventh mix (M7)**	**Eighth mix (M8)**	**Ninth mix (M9)**	Control **100% WF**
Quinoa	10	10	5	5	4	3	0	0	0	-
Red lentils	10	10	5	5	5	5	5	5	5	-
Pumpkin	20	30	35	40	45	50	60	70	75	-
Barley	10	15	15	10	20	20	5	5	4	-
Sesame	10	5	10	10	6	4	5	4	3	-
Teff	10	15	15	15	10	6	10	4	3	-
Red corn	10	5	5	5	3	4	5	4	3	-
Yellow corn	10	5	5	5	2	4	5	4	3	-
Millet	10	5	5	5	5	4	5	4	4	-
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Values in the same column are composite flour mixture. Mixtures = (M1-M9).

3.4 Determination of % gluten in composite flour

About 20 g of each composite flour sample was weighed into a Petri dish of known weight and thoroughly mixed with 1 ml of water to form dough. The dough is kneaded under running water to remove starch and later put into Petri dish and weighed. It was then dried in an oven (LCON53CF, Genlab, England) and weighed after drying (method 38-10, AACC 2000)[54]. The % gluten is calculated as follows:

$$\%\ \textbf{wet gluten} = \frac{\textbf{Weight of gluten}}{\textbf{Weight of orginal flour}} \times 100$$

3.5 Dough Preparation and bread making

Preliminarily, each of Quinoa, Red lentils, Pumpkin, Barley, Sesame, Teff, Red corn, Yellow corn, Millet and Wheat flour (control) was singly mixed with 5% refined sunflower oil, 2% commercial pressed yeast and 1% salt to produce ten different dough's (250g)[53]

The portions of dough were placed in lightly greased tins, held for another 30 min for final proofing, and then baked in the oven at 200°C for 50 min.

The breads were removed from oven and cooled to room temperature for 1 h prior to being packed in plastic bags that were sealed to prevent moisture loss.

Testing for sensory evaluation was done within 1 day after the breads had been removed from the oven and within 2 days for bread characteristics. Ten batches were produced and analyzed for each bread formulation (Figure 8).

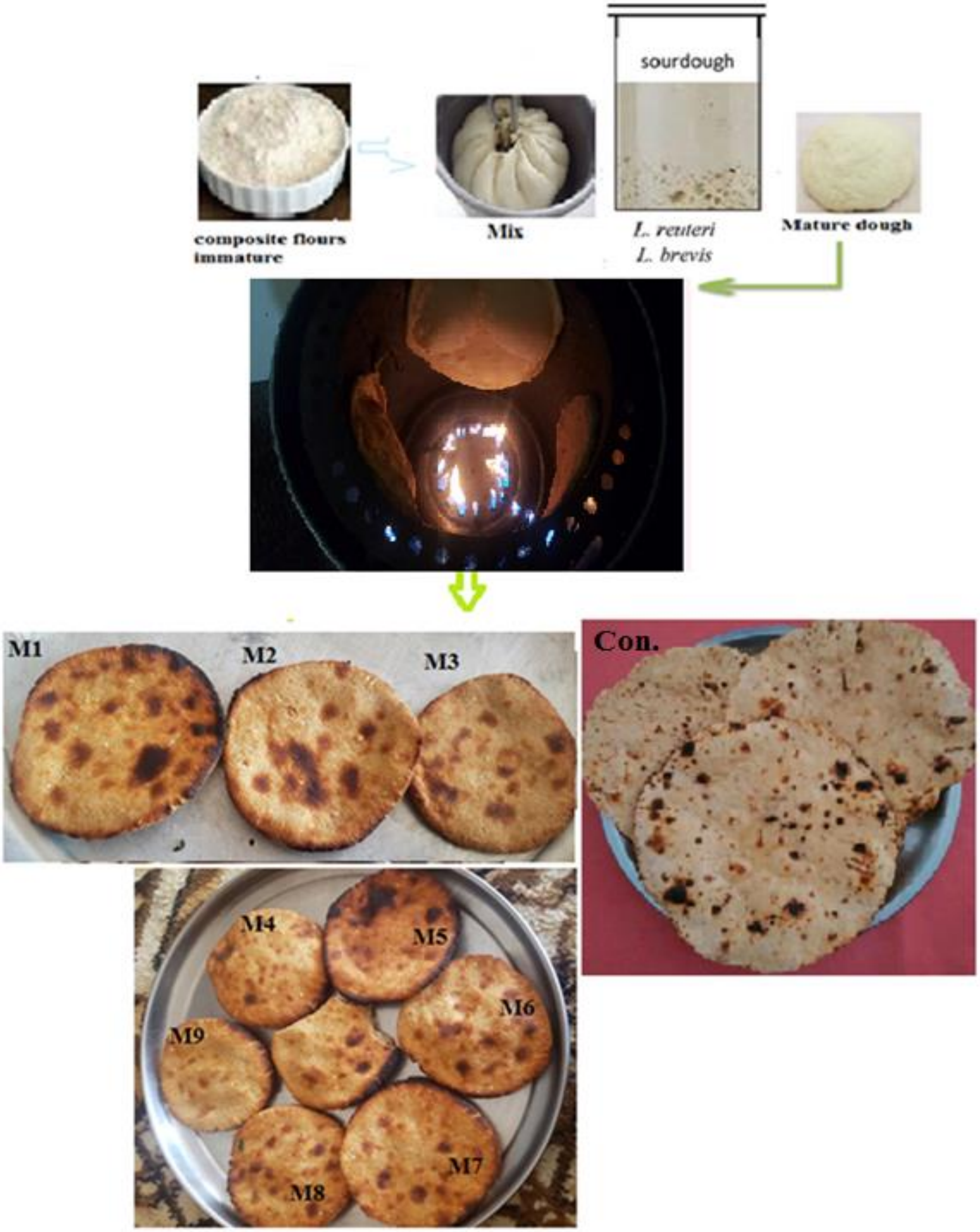


Figure 8 Dough Preparation and bread making from the sample of composite flours

3.6 Determination of the rheological properties of dough

Rheological properties were determined by using **Mixolab 2 - Dough Analysis (KPM, France)**. A 2.5 g of flour was dispersed in 25 mL water in an aluminum can and then the suspension was centrifuged at 160 RPM at the temperature of 50℃ for 1 min. The heating was then raised to

95℃ within 7.5 mins and held at 95℃ for 5 mins, and then cooled back to 50℃ within 7.5 mins and held at 50℃ for additional 2 mins[53] (Figure 9).

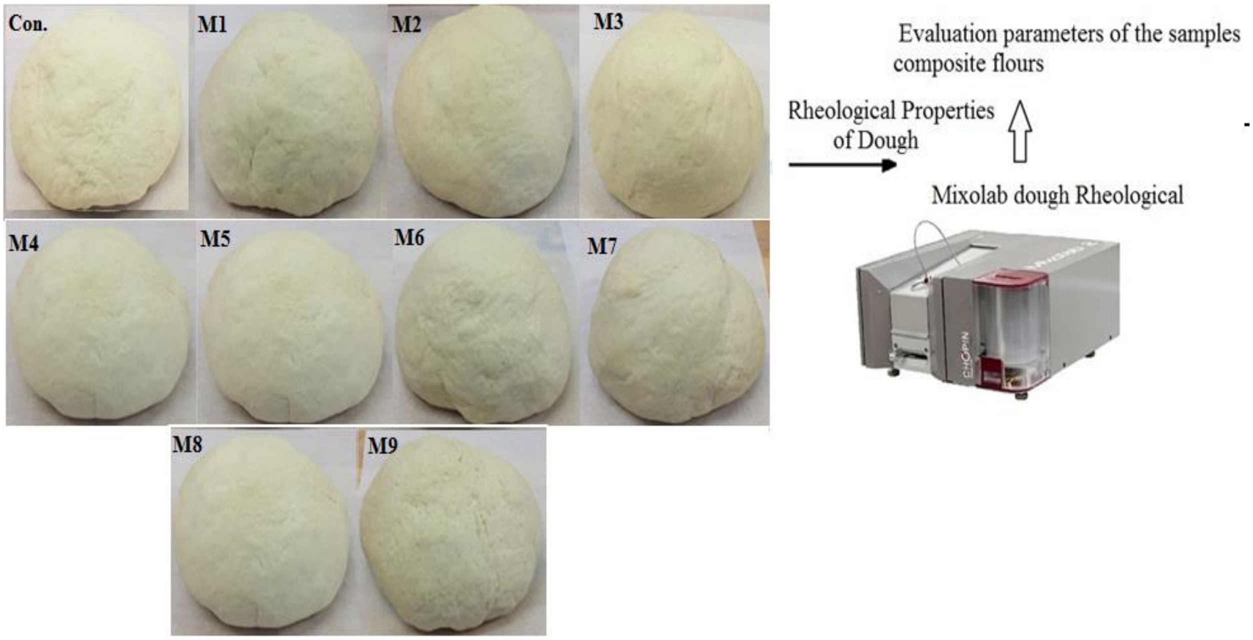


Figure 9 Dough preparation and bread baking of composite flours mixes

3.7 4.1 Proximate composition of the composite Flours

Moisture content was determined using AOAC 967.03[55]. The sample was dried at 105°C for 16 h in a draft air system (model UF55, Memmert Oven). The loss in weight was recorded as moisture. A conversion factor of 6.25 was used to convert total nitrogen to percentage crude protein. Ash content was determined by the method of Marsall 2010 [56] that involved burning off moisture and all organic constituents at 600°C in a VULCAN™ furnace (model 3-1750, Cole-Parmer). The weight of the residue after incineration was recorded as the ash content.

Fat content was determined by the method of AOAC 960.39 [57] using the Soxhlet extraction technique (model FOSS Soxtec™ extraction, Sweden). Nitrogen content was measured by the Kjeldahl method[58]. Carbohydrate content was calculated by subtracting the percentages of moisture, crude protein, ash and fat from 100. All measurements were carried out in triplicate.

3.8 Evaluation of Mineral contents (mg/100 g) of of composite flours

The concentration of selected minerals in the bread samples (brands), such as Na, K, Mn, Mg, Zn, Cu and Fe were determined using Flame Atomic Absorption Spectrophotometer vario- 6. The minerals were estimated after wet digestion of 5 g sample using concentrated HNO_3 and perchloric acid[59,]

3.10 Sensory Evaluation

Testing for sensory evaluation of taste, aroma, texture, appearance and overall acceptability was done within 1 day after the breads had been removed from the oven and within 2 days for bread characteristics[53]. Ten batches were produced and sensory evaluation for each bread formulation (Figure 8).

3.11 Microbiological analysis of composite flour

Microbiological analysis of the samples of composite flour was carrying out in the Collage of Science at Sana'a University, Yemen. The microbiological analysis included total plate count (TPC), total fungal count (TFC), total *coli* forms (TC), *Staphylococcus aureus*, anaerobic sulphate reducing bacteria, *Salmonella* sps., and *Vibrio parahaemolyticus* were done by (ISO 4833:1991)[60].

3.12 Statistical analysis

All determinations were performed in triplicate. The statistical analyses were conducted using either one-way or two-way ANOVA procedures depending on the experimental design. Statistical differences in samples were tested for at $p < 0.05$. Duncan's multiple-range test (DMRT) was used to differentiate between the mean.

4. Results and Discussions

4.1 Proximate composition of the composite Flours

The perspective of this current research was to formulate wheat substitutes from the locally available cereals and legumes.

The moisture, protein, lipids, ash, and carbohydrate contents of the composite flour samples are shown in Table 3.The moisture, protein, lipids, ash, and carbohydrate content of the composite flour samples ranged from 5.33-13.00 %, 2.7 -11.0 %, 1.0- 2.8%, 0.03-3.90% and 33.1- 81.4% respectively compared to the control. The table 3 showed significantly differences in moisture, protein, lipid, ash and carbohydrate content compared to the control was increasing. Significant reduction of moisture was observed in M3, M5 and M6 samples compared to the control. As insignificant reduction of moisture was observed in M1, M2, M7, M8 and M9 samples compared to the control. While the moisture content in M1 did not show any significantly difference with control. The rate of microbial (bacteria and molds) growth increases with moisture content from 13-15% in wheat, resulting in spoilage of the food[61]. The low moisture content of a product contributes to its increase shelf life[62]. The moisture content of all samples below 14 % was within moisture level recommended for storage stability of flours[63].

Table 3 showed significant differences in protein content compared to the control, as a significant decrease in protein was observed in samples No. 3 and sample No. 5. While sample No. 4 was the closest to the control sample (11%). Teff flour protein increases protein, fat and ash when added in compound flour, and reduces the carbohydrate content[63].

Compound bread that uses different types of legume flour is among the most studied alternatives to wheat flour, as previous research results[17-19] showed that partial or total replacement of wheat flour with legumes in bread making gives bakery products better amino acids, especially in lysine and threonine thus achieving adequate nutrition.

In our study, the addition of red lentil and pumpkin flour improved the volume, texture, and aroma of the compound bread to levels similar to the wheat sample (control). Asia and Africa where maize, which is known to lack tryptophan, being the staple food for those peoples, which led to reduced growth, poor bone growth, and neurological disorders[39] in those peoples, so in our study, it was proposed to add legumes such as red lentils and pumpkins in the compound flour mixture to provide health benefits from Through its antioxidant activity, lowering blood pressure, hypoglycemia, and hypocholesterolemia. , anti-hormone, anti-cancer, and other vital properties[43]. Legumes are rich in dietary fiber (8-28g/100g) and protein (21-37g/100g) [22]. The consumption of sorghum, barley (*Hordeum vulgare* L.), teff and millets has been linked to a multitude of health benefits, such as weight control[25], lowering serum cholesterol and triglycerides levels[27], reduction in starch digestibility and improvement of blood glucose control[24], and mitigation of gastrointestinal disorders including the risk of colon cancer [28].

The results of our current study on ash ratios and carbohydrates in Table 3 were according to the Yemeni standard specifications No. 22/2003[65].

In general, In general, sample No. 4 (M4) was the best in terms of its content of protein, fat, ash and carbohydrates because it corresponds to the recommended individual needs.

Table 3 Proximate composition of the composite Flours

Samples	**Moisture (%)**	(%) Protein	Lipids (%)	Ash (%)	Carbohydrates' (%)
Wheat flour (Control)	13.05 ± 0.0^{A}	12.7 ± 0.28^{B}	1 ± 0.02^{C}	0.32± 0.03^{D}	90.89
M1	13.00 ± 0.01^{A}	10.4 ± 0.08^{C}	2.4± 0.01^{A}	2.38 ± 0.03^{B}	58.3
M2	12.59 ± 0.12^{B}	9.02 ± 0.21^{D}	1.02 ± 0.11^{C}	2.7 ± 0.03^{B}	63.4
M3	6.09 ± 0.11 ^{G}	2.7 ± 0.22^{G}	0.1± 0.01^{C}	3.90±0.14^{A}	33.1
M4	10.34 ± 0.05^{D}	11 ± 0.13^{B}	2.0 ± 0.12^{B}	0.72 ± 0.02^{D}	50.0
M5	5.33± 0.01^{F}	3.0 ± 0.21^{F}	2.6 ± 0.22^{A}	1.44± 0.02^{C}	77.0
M6	9.30 ± 0.01^{E}	8.3 ± 0.01^{E}	2.8± 0.18^{A}	0.03± 0.0^{D}	70.1
M7	11.20 ± 0.13^{C}	8.3 ± 0.20^{E}	2.8 ± 0.07^{A}	0.68±0.03^{D}	81.4
M8	11.21 ± 0.12^{C}	8.3 ± 0.14^{E}	1.0 ± 0.15^{C}	0.66±0.03^{D}	77.0
M9	11.12 ± 0.19^{C}	8.3 ± 0.01^{E}	2.5±0.01^{A}	1. 8±0.03^{C}	80.3

Values with the same letters in the same column are significantly different. Control = 100% wheat flour (WF).

4.2 Evaluation of Mineral contents (mg/100 g) of of composite flours

In the present study, the content of seven mineral elements was analyzed: calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) and iron (Fe), as well as an additional two trace elements: zinc (Zn), copper (Cu) (mg/100 g db) and (for any formulations) and comparing them to the control.

The mineral contents of the composite flour samples in table 4 ranged from 257.68±3.39 - 755.04 ±0.13, 32.05±0.03 - 156.11±0.12, 37.15±1.87 - 168.88±2.99, 0.32±0.04 - 24.42±0.04, 92.88±2.81 - 415.13±1.10, 2.68±0.04 - 2.85±0.02 and 0.85±0.01- 0.88±0.04 respectively compared to the control. The mineral contents in Table 4 showed significantly differences in compared to the control where observed significant rise of Na in **M3, M6, M7, M8, M9** samples of composite flours compared to the control, while the M1, **M2, M4 and M5** samples were lower than the control.

The **M9** samples of calcium (Ca) were higher than the control, while samples from **M1-M8** were lower than control. The magnesium (Mg) content in the **M1** sample ranged 168.88±2.99 mg/100g compared to control, which is higher than the control, while the **M2 – M9** samples were lower than the control.

The iron (Fe) content in the M4 and M7 samples were higher than control, while the other samples showed in Table 4 were lower than the control.

The potassium (K), zinc (Zn) and copper (Cu) in all samples are higher than control Consumption of the usual amount of composite bread per day satisfies only 3.05% of the recommended daily intake of iron[66].

The importance of these results is connected with the fact, that iron deficiency adversely affects the physical growth of school- aged children. Iron deficiency anemia affects >1.2 billion individuals worldwide and iron deficiency in the absence of anemia is even more frequent[67]. The iron content in pumpkin seeds is 23.97 mg/100g, and after roasting its amount decreases by 25.60%[68].

Our results are very close to the findings of El-Demery and Lotfy (2015)[69] and when referring to Yemeni Standard Specifications no. 22/2003[65] which determined the percentage of iron in adult foods that are mainly made from grains and pulses, as a minimum of 24.0 mg per 100 grams of the product on the basis of dry weight, and this percentage is identical with the results of our research.

Recommended dietary allowance (RDA) for adult individual of Na is 1500mg, Ca is 1000 mg, Mg for F is 310mg and M is 400 mg, Fe for F is 18 mg, and M is 8 mg, K is 4700mg, Zn for F 8mg and M is 11mg, Cu is 900 mg. M9 is the nearest sample to the RDA in Na, Ca, K, M8 is the nearest sample to the Zn while M8, M2 are the nearest to the Cu, M1 is the nearest sample to the Mg and M4 is the nearest sample to the Fe. Sodium, calcium, magnesium, ferritin, potassium, zinc, copper content of the composite flour samples is shown in table 4.

Table 4 Mineral contents (mg/100 g db) of formulated complementary flours.

Element	control	M1	M2	M3	M4	M5	M6	M7	M8	M9
Na	536.26±7.27^{D}	482.22±2.02^{E}	488.28±1.04^{E}	754.22±1.12^{A}	257.68±3.39^{F}	486.40±3.59^{E}	691.04±3.31^{C}	701.01±1.11^{B}	751.03±1.10^{A}	755.04 ±0.13^{A}
Ca	141.67±1.78^{B}	59.89±2.58^{G}	83.68±2.99^{E}	112.59±0.26^{D}	32.05±0.03^{H}	62.43±2.13^{F}	107.24±1.01^{D}	111.14±0.76^{D}	131.10±0.12^{C}	156.11±0.12^{A}
Mg	138.80±2.58^{B}	168.88±2.99^{A}	75.98±2.99^{D}	37.15±1.87^{G}	59.47=±1.78^{F}	64.74±1.76^{E}	75.86±2.31^{D}	88.86±2.31^{C}	90.07±1.01^{C}	92.14±1.01^{C}
Fe	4.69±0.27^{B}	0.42±0.04^{E}	1.40±0.07^{C}	1.74±0.17^{C}	4.14±0.10^{B}	1.72±0.10^{C}	0.32±0.04^{F}	24.42±0.02^{A}	0.44±0.02^{E}	0.53±1.12^{D}
K	14.07±5.34^{F}	240.16±0.32^{C}	92.88±2.81^{E}	139.59±2.60^{D}	97.11±2.25^{E}	253.63±0.13^{B}	410.49±2.23^{A}	414.49±1.13^{A}	417.19±1.13^{A}	415.13±1.10^{A}
Zn	2.50±0.22^{B}	2.69±0.03^{A}	2.68±0.04^{A}	2.74±0.07^{A}	2.68±0.05^{A}	2.69±0.04^{A}	2.72±0.08^{A}	2.72±0.01^{A}	2.85±0.02^{A}	2.77±1.02^{A}
Cu	0.52±0.04^{B}	0.86±0.04^{A}	0.88±0.04^{A}	0.85±0.01^{A}	0.85±0.02^{A}	0.86±0.03^{A}	0.87±0.04^{A}	0.86±0.04^{A}	0.88±0.01^{A}	0.87±3.01^{A}

Values with the same letters in the same row are not significantly different

4.3 Gluten composition of composite flours

The percentage wet gluten content of the composite flours is presented in Table 5. It ranged from 14.10 to 24.34%. The highest wet gluten is present in the M7 substitution (24.70%) while the lowest was in the M3 substation (14.00%) when compared with the control 24.90%. Gluten content slight decreased as the level of substitution increased. Dough from M1 - M6 flours were elastic during kneading and washing under water, thus enabling more washing of the starch from gluten fraction. As expected, the yields of gluten fraction were closely associated with protein contents of their flours (based on values in Table 6). Having examined the protein content (Table 6) and gluten content (Table 5) of the composite flours, it is assumed that these flours (M1–M9) could be substituted into wheat flour and the composite flours still maintaining its protein nature for better rheological and higher dough formation. Past research works[70] have also proved this statement to be true on substitution of wheat flour with locally corps to form composite bread products and other. The addition of wheat gluten or bread improvers that mimic the viscoelastic and gas retention properties of gluten, such as hydrocolloids, emulsifiers, and enzymes, is the most straightforward and widely used method in composite bread making. For example, the composite bread containing 61.8% barnyard millet, 31.4% wheat, and 6.8% gluten exhibited comparable textural and sensory attributes to refined wheat bread[71].

Table 5 Gluten content (%) of composite flour samples (Mixers).

Samples	% Gluten
M1	17.54 ± 0.13^{C}
M2	15.60 ± 0.14^{E}
M3	14.10 ± 0.05^{G}
M4	**24.34 ± 0.10^{A}**
M5	16.70 ± 0.05^{D}
M6	15.30 ± 0.12^{F}
M7	23.70 ± 0.12^{B}
M8	23.20 ± 0.11^{B}
M9	23.10 ± 1.20^{B}
Control	24.70 ± 0.10^{A}

Values with the same letters in the same column are not significantly different.

4.3 Rheological properties of the dough

The data of the composite flours are summarized in Table 6. The protein content of the flours ranged from 9.51 to 10.61% with samples M1 and M5 and M8 not significantly different but they have significantly different with each other sample. The protein content of the substituted flours decreased with increasing substitution.

The moisture content of the substituted flours ranged from 12.71 to 13.71% and that of control is 13.32%, water absorption of the composite flour dough ranged from 60.52 to 72.31% and was significantly higher than the control (57.81%). The absorption of more water during mixing is a typical characteristic of composite starches[72]. Several studies also reported that the dough made from composite flour absorbed more water than that made from wheat flour alone[73, 74]. The amount of water absorption of the flour samples, according to Table 6 increased with every increment of flour blends. Water absorptions were monitored up to calibrated points on the burette at which the dough became sticky.

The arrival time of dough made from the substituted flours (M1 - M9) was the same with control (about 1 min), while the stability time of these dough ranged from 7.71 to 8.00 minutes compared to control (7.71 min). Development time is the time from the first addition of water to the time the dough reaches the point of greatest torque. During this phase of mixing, the water hydrates the flour components and the dough is developed. There was no significant difference in the development time between the dough with or without substitution (1.50 to 1.53 min). This shows that the composite flours, though with different water absorption, started forming and developing during mixing as recorded by dynamometer on a kymograph chart. The level of substitution therefore does not appear to affect the arrival and development time of the composite flours.

Table 6 **Rheological properties of the dough from composite flour mixers.**

Mixes	Protein (%)	Moisture (%	Water absorption (%)	Arrival time (min)	Development time (min)	Dough stability time (min)
Control	10.61 ± 0.02^{A}	13.32 ± 0.12^{B}	57.81 ± 0.01^{G}	1.00 ± 0.03^{A}	1.53 ± 0.02^{A}	7.71 ± 0.04^{D}
M1	10.59 ± 0.01^{A}	13.41 ± 0.13^{A}	62.80 ± 0.00^{E}	1.00 ± 0.02^{A}	1.50 ± 0.01^{A}	7.60 ± 0.02^{D}
M2	9.92 ± 0.01^{D}	13.42 ± 0.22^{A}	67.31 ± 0.00^{B}	1.24 ± 0.01^{A}	1.50 ± 0.02^{A}	8.00 ± 0.01^{C}
M3	9.51 ± 0.04^{E}	13.42 ± 0.21^{A}	72.31 ± 0.01^{A}	1.24 ± 0.02^{A}	1.52 ± 0.03^{A}	7.71 ± 0.01^{A}
M4	10.21 ± 0.03^{B}	13.11 ± 0.02^{C}	60.52 ± 0.01^{F}	1.07 ± 0.01^{A}	1.51 ± 0.02^{A}	12.01 ± 0.01^{A}
M5	10.51 ± 0.01^{A}	12.71 ± 0.16^{D}	63.61 ± 0.01^{D}	1.00 ± 0.02^{A}	1.51 ± 0.01^{A}	10.01 ± 0.00^{B}
M6	10.02 ± 0.02^{C}	12.72 ± 0.01^{D}	66.21 ± 0.01^{C}	1.25 ± 0.01^{A}	1.52 ± 0.02^{A}	8.02 ± 0.02^{C}
M7	10.21 ± 0.01^{B}	13.11 ± 0.02^{C}	60.54 ± 0.02^{F}	1.11 ± 0.01^{A}	1.53 ± 0.02^{A}	12.01 ± 0.00^{A}
M8	10.51 ± 0.02^{A}	12.71 ± 0.06^{D}	63.52 ± 0.02^{D}	1.00 ± 0.00^{A}	1.51 ± 0.03^{A}	10.02 ± 0.01^{B}
M9	10.02 ± 0.02^{C}	12.71 ± 0.01^{D}	66.10 ± 0.01^{C}	1.25 ± 0.00^{A}	1.50 ± 0.01^{B}	8.01 ± 0.01^{C}

Values with the same letters in the same column are not significantly different. Control = 100% wheat flour (WF).

4.6 Sensory evaluation of bread samples

Germinated Quinoa, Red lentils, Pumpkin, Barley, Sesame, Red corn, Yellow corn and Millet flours mixture were the main ingredients, and we formulated nine samples (M1 to M9) with different proportions of them and compared all samples to control (wheat flour 100%). A trained panelist with ten members then examined the sensory attributes of nine samples in addition to the control sample according to the 5-point hedonic scale. The sample M7 received the highest overall acceptance score among them.

The Taste, Flavor, Texture, Appearance, and Overall accept of the composite flour samples ranged from 1.12-3.94, 1.53 – 3.91, 1.84-3.90, 3.74-1.37, 1.1-3.50 respectively compared to the control (Table 7).

This suggests that bread from wheat flour substituted with Quinoa, Red lentils, Pumpkin, Barley, Sesame, Red corn, Yellow corn and Millet flour mixture (Table 2) not significantly affect desirable sensory attributes of the bread. Olaoye and Onilude (2008)[75] in their report also affirmed this by suggesting that Teff, Quinoa, Red corn and Yellow corn flour mixture up to 10% level will give bread similar to the sensory characteristics of wheat flour.

This suggests that the quality of bread that can be produced from composite flour mixture (Table 2) depends on the level of substitution. This observation is consistent with previous reports by Eddy et al. (2007)[76] that observed changes in the quality of bread produced from cassava-wheat composite flours at different levels of substitutions.

The enrichment of wheat bread with legume like red lentils and pumpkin at levels greater than 10% will lead to acceptable flavors for consumers[62].

All the formulations were evaluated for their acceptability by trained panelists using a five point hedonic scale. Although, many formulations were found to be organoleptically acceptable recording moderately to extremely like scores, generally formulations M 4 were highly acceptable by panelists and scored significantly ($P < 0.05$) higher than the other wheat substitutions samples. Their mean score ranged between 3.94 to 3.50 in terms of taste, flavor, texture, appearance and overall accept.

Table 7 Sensory evaluation of composite bread samples

Samples	Taste	Flavor	Texture	Appearance	Overall accept
Control	4.12^{A}	4.22^{A}	4.33^{A}	4.24^{A}	4.06^{A}
M1	2.16^{C}	2.66^{C}	2.37^{C}	1.37^{D}	1.47^{C}
M2	2.17^{C}	2.72^{C}	1.84^{D}	1.63^{D}	1.32^{C}
M3	2.15^{C}	2.83^{C}	2.26^{C}	1.56^{D}	1.33^{C}
M4	3.94^{B}	3.91^{B}	3.90^{B}	3.74^{B}	3.50^{B}
M5	2.19^{C}	2.84^{C}	2.26^{C}	1.63^{D}	2.26^{C}
M6	2.19^{C}	2.74^{C}	2.37^{C}	2.50^{C}	2.32^{C}
M7	2.15^{C}	2.63^{C}	1.85^{C}	1.52^{D}	2.34^{C}
M8	2.12^{C}	2.80^{C}	2.35^{C}	2.54^{C}	2.30^{C}
M9	1.12^{C}	1.53^{C}	2.31^{C}	2.53^{C}	1.1^{D}

Values with the same letters in the same column are not significantly different.

4.7 Microbiological parameters

The microbial analysis for all composite flour mixture were ascertained through assessment of microbial parameters including total plate count (cfu/g), total coliforms, (MPN/g), *E. coli* (MPN/g), *Staphylococcus aureus* (cfu/g), Anaerobic sulphate Reducing bacteria at 35^{0}C (cfu/g), *Salmonellaspp.* (Occurrence in 10 g), *V. parahaemolyticus* (occurrence in 10 g) and total fungal count (cfu/g) which is indicated in Table 8. All the microbial parameters in both products were shown within the acceptable limits of WHA/FAO standards[61], Since both the products comply with microbial safety guidelines, finally it is said that they are absolutely fit for consumption immediately after cooking.

Table 8 Microbiological analysis of composite flour simples (mixes)

Parameter	**Standard**	Results of analysis									
		M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
Total plate count (cfu/g)	**$5x10^{4}$**	$1.1x10^{2}$	$3.3x10^{2}$	$1.1x10^{2}$	$1.1x10^{2}$	$3.1x10^{2}$	$1.1x10^{2}$	$1.0x10^{3}$	$1.1x10^{2}$	$1.0x10^{3}$	$2.0x10^{2}$
Total coliforms at 37^{0}c (MPN/g)	**5**	<2	<2	<2	<2	<2	<3	<2	<3	<3	<3
E.coil (MPN/g)	**5**	<3	<2	<3	<2	<2	<3	<2	<3	<2	<2
Staphylococcus aureus (cfu/g)	**$<2x10^{2}$**	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Salmonella species (occurrence/10g)	**Absent in 10**	Absent in 10	Absent in 10	Absent in 10	Absent in 10	Absent in 10	Absent in 10	Absent in 10	Absent in 10	Absent in 10	Absent in 10
Vibrio parahaemolyticus (occurrence/10g)	**$<10^{3}$**	Absent in 10	Absent in 10	Absent in 10	Absent in 10	Absent in 10	Absent in 10	Absent in 10	Absent in 10	Absent in 10	Absent in 10
Total fungal count (cfu/g)	**$<2x10^{3}$**	$2.3x10^{2}$	$2x10^{2}$	$1.5x10^{2}$	$1.4x10^{2}$	$1.0x10^{2}$	$2.0x10^{2}$	$1.2x10^{2}$	$1.3x10^{2}$	$1.4x10^{2}$	$1.3x10^{2}$

Conclusions

1. Findings in this study have shown the potential for the production of bread of acceptable quality from Quinoa, Red lentils, Pumpkin, Barley, Sesame, Red corn و Yellow corn and Millet flours mixture
2. From the present study it may be inferred that Sesame, Pumpkin and Red lentils could be added to bread up to levels of 40% without significant adverse effects regarding the crust color, crumb structure and uniformity.
3. It was thus concluded that bread produced from mixtures of 5% Red lentils, 60% Pumpkin, 5% Barley, 5% Sesame, 10% Teff, 5% Red corn, 5% Yellow corn and 5% Millet gave the best products due to its protein, mineral and lesser anti-nutrient contents as well as their acceptability to the consumers.
4. In any case, consumption of bread samples made of Quinoa, Red lentils, Pumpkin, Barley, Sesame, Red corn و Yellow corn and Millet flours mixture could be said to be more beneficial in terms of improving the nutritional status of the consumers because price the bread which made of wheat flour is very high in Yemen.
5. In this study, we have discussed the benefits and challenges associated with composite bread making using various types of locally wheat flour substitutes, the demand and research of which will likely increase exponentially in the near future.

Recommendations

1. A further study on the dynamic rheological properties of composite flour mixture dough and bread characteristic.
2. A further study on the bread quality characteristic, including Loaf weight, loaf Shape, Crust color and Crumb color.
3. A further study on the commercial bread improvers
4. A further study on locally available natural bread improvers (e.g., fruits) rich in ascorbic acid that acts as an oxidizing agent in strengthening the gluten.
5. Going forward, interdisciplinary approaches addressing the current knowledge gaps in the environmental, nutritional, health and technological dimensions are required. The synergism between sustainability diet, human nutrition, microbiomics and food science is necessary to scale up research results for large-scale positive impact

6. A further study on characterize the microbial communities in different fermented foods and identify specific microbes responsible for their unique flavors and aromas.

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التركيبة الغذائية المثلى في صناعة الخبز بالاعتماد على بدائل القمح المستدامة محليًا

الملخص بالعربي

يعتبر الخبز هو الغذاء الأساسي المفضل في اليمن ، ويتم إنتاجه بشكل تقليدي من القمح (*Triticum aestivum*) وبسبب ارتفاع الطلب وانخفاض الإنتاج المحلي ؛ يتم استيراد حوالي 95 ٪ من القمح المطلوب من بعض الدول مثل أستراليا وروسيا وأوكرانيا والولايات المتحدة الأمريكية والهند بقيمة تصل إلى حوالي سبعمائة مليون دولار سنويًا، وبسبب التكاليف المتزايدة للقمح المستورد وعدم القدرة على استدامة واردات القمح الوطنية لصناعة الأطعمة القائمة على القمح، يجعل من الضروري استبدال القمح بمحاصيل محلية أخرى.

في هذه الدراسة ، تم تحضير تسع تركيبات من الخبز عن طريق الاستبدال الكلي لبعض المحاصيل المحلية مثل دقيق الكينوا والعدس الأحمر واليقطين والشعير والسمسم والذرة الحمراء والذرة الصفراء والتيف ودقيق الدخن لتقييم صناعة الخبز المركب بالمقارنة مع دقيق القمح (عينة المقارنة).

أضهرت الفحوصات الكيميائية والريولوجية لكل العينات أن العينة رقم 4 (M4) هي افضل عينة في محتوى المغذيات، كما أوضحت الدراسة بأن الأحياء الميكروبية في جميع العينات ضمن الحدود المقبولة لمعايير منظمة الصحة العالمية ومنظمة الأغذية والزراعة، وبالتالي تكون العينات صالحة للاستهلاك البشري مباشرة بعد الطهي.

وتم تقييم جودة جميع تركيبات الخبز المحضرة من أجل قبولها من قبل أعضاء اللجنة المدربين باستخدام مقياس المتعة من أربع نقاط، على الرغم من أن العديد من التركيبات كانت مقبولة من الناحية الحسية وسجلت درجات متشابهة إلى حد كبير ، إلا أن التركيبة M4 كانت مقبولة بشكل كبير من قبل أعضاء اللجنة وسجلت درجات أعلى بشكل ملحوظ ($P < 0.05$) من عينات بدائل القمح الأخرى، حيث تراوح متوسط درجاتهم بين 3.50 إلى 3.94 من حيث المذاق والنكهة والملمس والمظهر والقبول العام.

بشكل عام: أظهرت هذه الدراسة بأن المحاصيل المحلية الموضحة في الجدول (2) مناسبة لتطوير الخبز ذوي الجودة التكنولوجية الجيدة والمظهر التغذوي المحسن ، مما يضيف قيمة إلى طحين الأجداد غير المستخدم.

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التركيبة الغذائية المثلى في صناعة الخبز بالاعتماد على بدائل القمح المستدامة محليًا

اطروحة مقدمة كاستيفاء جزئي لمتطلبات الحصول على درجة البكالوريوس في التغذية العلاجية والحميات

إعداد:

1- سمية المعلمي
2- الهام العماري
3- عُلا قيران
4- صفاء الأنسي
5- هديل العسكري
6- حنان الأهنومي
7- هديل التتار
8- هلا البيضاني
9- اسماء هبه
10- الشيماء خيران
11- الاء الطباطبي

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2022م