Yeast derived metabolites and their impact on nutritional and bioactive properties of African fermented maize products

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ABSTRACT

Fermented foods are an integral part of the diet for many communities in Africa. The fermentation of maize is characterized by an improved nutrient content/bioavailability and the presence of bioactive compounds which are largely attributed to the activity of the microbial population mainly lactic acid bacteria (LAB) and yeasts. The role of LAB in maize and other cereal fermentations has been extensively studied. However, limited literature is available on the role of yeasts in maize fermentations and resultant nutritional and health promoting properties. This review explores the contribution of yeasts to the nutritional and health properties of fermented maize and other cereal products. Additionally, the proliferation and growth characteristics of dominant yeasts and their derived metabolites are discussed. It is particularly important to comprehend the processes and type of yeasts involved in maize fermentation as an essential step in maize product development. This will facilitate the development of yeast-based starter culture, improve maize fermentation process and provide better understanding of associated nutrition and bioactive properties.

Keywords: African Maize Products, Yeast Fermentation, Bioactive metabolites.

1. INTRODUCTION

Maize (*Zea mays L.)* is a staple crop that constitutes 38% of food supply in the African continent and provides about 357.67 kcal/capita/day (Guyot, 2012; Palacios-Rojas et al., 2020; Ranum et al., 2014). Consumption of maize is mainly as fermented thick or thin porridges or alcoholic and non-alcoholic beverages in SubSaharan Africa. Maize fermentation is dominated by LAB (lactic acid bacteria) at a population of 10^7–10^{11} CFU/g or mL of food followed by a yeast population of 10^4-10^6 CFU/g or mL of food (Table 1) (Chaves-López et al., 2020; Greppi et al., 2013). LAB genera involved are predominantly *Pediococcus*, *Streptococcus*, *Lactococcus* and some *Lactiplantibacillus* species which are homo-fermentative and hetero-fermentative species of the genera *Weisella*, *Leuconostoc* and *Lactobacilli* (Chelule et al., 2010). Amylolytic lactic acid bacteria (ALAB) represent about 10% of the LAB population and are able to hydrolyse crude starch to fermentable sugars by action of inherent α-amylases (Guyot, 2012). Yeasts on the other hand assimilate or utilize carbon and nitrogen substrates such as organic acids, amino acids to generate volatile and non-volatile metabolites (Fleet, 2007). The yeast population is responsible for the production of volatile compounds such as alcohols, esters, aldehydes and ketones (Chaves-López et al., 2020), and bioavailability of nutrients (Johansen et al., 2019).

Maize is also a valuable source of bioactive compounds known to exert health promoting properties. These compounds include phenolics and carotenoids found predominantly in yellow maize, and anthocyanins in blue maize (Ekpa et al., 2019). Like other cereal grains, nutrients present in maize are low quantitatively and not readily available for digestion and absorption which affects its contribution to the nutrition and well-being of communities who depend on maize as a staple food. This is attributed to the presence of antinutrients such as phytic acid (phytate), tannins and polyphenols. The main antinutrient in maize is phytate found between 0.75% and 2.22% constituting 60-90% of the kernel phosphate (Palacios-Rojas *et al*., 2020). The negatively charged phosphate groups in phytate chelate multivalent-cation minerals such as calcium, iron and zinc; thus affecting their bioavailability (Nuss and Tanumihardjo, 2010).

Fermented maize products (alcoholic and non-alcoholic beverages or food (Franz et al., 2014) are unique based on the regional food processing practices (method of processing, duration of fermentation and/or addition of other substrates (Gadaga et al., 1999; 2013, 2021; Simatende

Table 1: African maize based fermented foods (adapted from Johansen et al., 2019; Chaves-López et al., 2020)

Non-alcoholic beverages						
Food product	**Country of origin**	**Associated yeast species** *dominant (>50% of the population)	**Yeast count (Log_{10} CFU/g or Log_{10} CFU/mL)**	**Fermentation period and temperature**	**pH**	**Reference**
Ogi/Akamu/Koko	Nigeria, Benin, East Africa, Ghana	*C. tropicalis**, *Candida krusei*, *Geotrichum fermentans*, *Geotrichum candidum*, *Rhodotorula graminis*, *S. cerevisiae*, *Candida albicans*, *Candida utilis*, *Clavispora lusitaniae*, *Rhodotorula*	2.9-7.9	72 h; 28-30°C	3.2-4.1	(Adesulu-Dahunsi et al., 2020; Chaves-López et al., 2020)

		glutinis, Saccharomyces pastorianus				
Ogi/Uji	Nigeria, Benin, East Africa	*C. tropicalis*, C. krusei, C. tropicalis, G. fermentans, G. candidum, Rhodotorula graminis, S. cerevisiae, Candida albicans, C. krusei, C. tropicalis, C. utilis, C. lusitaniae, G. candidum, G. fermentans, Rhodotorula glutinis, R. graminis, S.*	2.9-7.9	24-48 or 72 h; 28-30°C	3.8-4.1	(Chaves-López et al., 2020; Gabaza et al., 2017; Johansen et al., 2019)

		cerevisiae, S. pastorianus				
Munkoyo	Zambia	*S. cerevisiae*		24-4 h; 25-30°C	3.3-4.0	(Chaves-López et al., 2020; Phiri et al., 2019)
Togwa	Tanzania	*C. tropicalis, P. kudriavzevii*, C. glabrata, Hanseniaspora guilliermondii, I. orientalis, P. anomala, P. burtonii, P. guilliermondii, P. kudriavezvii, P. norvegensis, K. marxianus, S. cerevisiae, C. pelliculosa, C. tropicalis,*	5.0-7.0	12-24 h; RT	3.1-3.3	(Chaves-López et al., 2020; Gabaza et al., 2017; Johansen et al., 2019)

		Kluyveromyces marxianus, Pichia anomala, *S. cerevisiae*				
Solid food						
Kenkey	Ghana	*P. kudriavzevii*, S. cerevisiae; Candida kefir, C. krusei, C. mycoderma, C. tropicalis,*	2.7-6.2	2-4 days; RT	3.7	(Chaves-López et al., 2020; Johansen et al., 2019)
Gowé	Benin	*C. tropicalis*, Kluyveromyces marxianus*, P. kudriavzevii*, W. anomalus, Candida krusei*	4.9-6.2	16 h; RT	3.6-4.1	(Chaves-López et al., 2020; Johansen et al., 2019)
Dough						

Mawè	Benin, Togo	*W. anomalus*, K. marxianus*, P. kudriavzevii*, S. cerevisiae*, C. glabrata, C. tropicalis, Candida krusei, Clavispora lusitaniae,*	4.5-8.0	1-3 days	4.2	(Chaves-López et al., 2020; Houngbédji et al., 2018; Johansen et al., 2019)
Low alcoholic beverage						
Sekete		*Geotrichum* spp., *Saccharomyces* spp., *S. cerevisiae*	2.24	2-3 days, RT	2.8-4.3	(Chaves-López et al., 2020; Sanni and Lönner, 1993)

RT = room temperature

et al., 2015). The non-alcoholic fermented food products such as mahewu, incwancwa, ogi (Akamu), togwa, borde, munkoyo, uji and kunu are consumed as part of the diet in communities (Mashau et al., 2021). Thin fermented maize porridges or gruels are mainly used as weaning food for infants e.g. ogi (Blandino et al., 2003), incwancwa (Chelule et al., 2010) and beverages such as munkoyo and togwa (Mashau et al., 2021). While stiff and soft porridges and beverages for consumption are also prepared by fermenting a mixture of maize and water for one to three days e.g. kenkey (Blandino et al., 2003). Whereas beverages such as mahewu, maxau, munkoyo, borde and togwa are non-alcoholic and consumed as refreshments and as part of meals (Mashau et al., 2021; Misihairabgwi and Cheikhyoussef, 2017).

These fermented foods are reported to provide nutritional (McKevith, 2004) and health benefits to the consumer (Bell et al., 2018; Şanlier et al., 2019). The effect of fermentation on the nutritional value of food is variable although improvements are substantial (Blandino et al., 2003, Nout, 2009). This variability is attributed to either substrate, microbial population or fermentation conditions. The substrate used and the microorganism(s) involved in the conversion of the substrate are the two primary driving factors in a fermentation. Both these factors interact in unison; the activity of microorganisms induces modifications on the substrate while the substrate provides nutrients for the microorganism(s) (Tamang et al., 2016a, 2016b). In this food ecosystem there is no shortage of substrates and microorganisms are ubiquitous, thus a hub of microbial interactions and substrate modifications exists resulting in a myriad of metabolites. Through the monitoring of microbial diversity and activity, and substrate modification; studies on understanding the role of fermentation in food ecosystems have emerged (Gabaza et al., 2017; Galati et al., 2014; Nout, 2009; Nout and Motarjemi, 1997; Şanlier et al., 2019). Notably, fermentation functions in the enrichment of the food through the development of flavours, aromas and textures of food substrates; release of essential minerals; preservation of food through the production of organic acids; enrichment of food substrates with protein, essential amino acids, fatty acids and vitamins; detoxification of the food; decrease cooking time and fuel requirements (Gabaza et al., 2017).

Metabolic processes of microorganisms during fermentation lead to decreases in inhibitors of digestive enzymes (such as trypsin and amylase), and other anti-nutrients such as phytate and tannins. The production of metabolic compounds is attributed to the enzymatic activity of the microorganisms present and thriving in the ferment. In general, fermentation involves a mixed microbial population of bacteria, yeasts and fungi (Smid and Lacroix, 2013). The microbial interactions during fermentation account for several changes to the substrate based on their

unique metabolic activity thus affecting overall product quality. Literature is flooded with studies on lactic acid bacteria and their role in cereal fermentations. However, very scarce literature is available on the role of yeasts in cereal and specifically maize fermentations. This review seeks to understand the contribution of yeasts in the nutritional and health properties of fermented maize foods.

2. YEAST ABUNDANCE IN AFRICAN FERMENTED MAIZE FOODS

Yeasts such as *Saccharomyces cerevisiae*, *Pichia kudriavzevii*, *Candida* spp. and *Kluyveromyces* spp. dominate ($\geq$50%) maize fermentations (Table 1) (Houngbédji et al., 2018; Johansen et al., 2019; Tamang et al., 2016b) and this is attributed to their inherent growth requirements (Cuvas-Limon et al., 2021). Traditional methods to produce maize products include soaking, germination, wet grinding, sieving and/or fermentation (Palacios-Rojas et al., 2020). These methods are mainly applied at household level as an artisanal food processing method of wet-milling and/or wet-sieving. Here, maize is steeped in either an earth-ware, plastic or metal pot for 1-3 days by mixing 1 part of maize with 2/3 parts water to yield a slurry. This functions to hydrolyse the starch by endogenous grain amylases to release fermentable sugars as substrate for microorganisms during fermentation (Chaves-López et al., 2020) in addition, amylolytic LAB are also activated to hydrolyse starch (Guyot 2012).

Identification of the microbial population of African fermented foods prior to a decade ago relied on culture dependent methods. However, the broad application of culture-independent methods has allowed the identification of species present in minute concentrations and to an extent species under the viable but non-culturable state. Greppi et al. (2013) identified a yeast population of 3.75, 5.52 and 4.40 Log_{10} CFU/g in the products ogi, mawè and gowé, respectively using a culture-dependent method. In comparison, Hounhouigan et al. (1994; 2018) reported a yeasts population of Log_{10} 4.4 CFU/g at time zero to Log_{10} 7.5 CFU/g at the end of fermentation. Using Denaturing Gradient Gel Electrophoresis (DGGE) analysis, the following species were identified *P. kudriavzevii* (teleomorph of *Candida krusei*), *Clavispora lusitaniae*, *S. cerevisiae*, *Dekkera bruxellensis*, *Kluyveromyces marxianus* and *Debaryomyces hansenii*. Notably, the species *D. bruxellensis* and *K. marxianus* were not detected by culturing but by DGGE analysis of the DNA from samples. This highlights the limitation of the culture-based method. In all the samples *C. krusei* was the abundant species accounting 69% of all the species present in the products (Greppi et al., 2013). The use of rRNA sequencing on maize togwa detected the following yeast species - *Candida glabrata*, *Pichia anomala*, *Pichia*

guilliermondii and *K. marxianus* (Hellström et al., 2010). Hounhouigan et al. (1994) also identified *C. krusei* as the dominant yeast species in mawè product. During fermentation, *S. cerevisiae* was reported to be Log_{10} 6 CFU/g after a period of 24-48 h; the steeping and early phases of fermentation (Jespersen et al., 2003). A similar trend was observed by Omemu et al. (2007) with *S. cerevisiae* being frequently isolated in the steeping period. The growth of *S. cerevisiae* in the early stages is partly attributed to the availability of maltose a major carbohydrate in cereals which serves as a substrate for yeast growth. For example, 98% of yeast isolates in fermented maize dough were found to be able to assimilate maltose, DL-lactate and melibiose. Of the *S. cerevisiae* isolates 100%, 73% and 38% possessed the *MAL11*, *MAL31* and *MAL61* genotype, respectively which encodes the maltose permease enzyme (Jespersen, 2003).

As the acidity increases during fermentation, the total yeast population varies and non-*Saccharomyces* yeasts such as *C. krusei*, *C. tropicalis*, *Geotrichum fermentans* and *Geotrichum candidum* were identified (Omemu et al., 2007). This is attributed to the tolerance levels of non-*Saccharomyces* yeasts to stress factors. Using pyrosequencing, Mendoza et al. (2017) reported on *S. cerevisiae* as the most abundant species followed by species from the genera *Kluyveromyces*, *Pichia*, *Debaryomyces* and *Candida*. This method allowed for the grouping on the identified species based on their proliferation at the different stages during fermentation. This then provides informed tracking and contribution of the yeast species during fermentation. Currently, there is lack of data on the application of pyrosequencing in African fermented foods.

3. MICROBIAL INTERACTIONS DURING MAIZE FERMENTATION

In fermented foods, yeasts and LAB exist in a mutualistic interaction (Smid and Lacroix, 2013) and synergistic interaction (Adesulu-Dahunsi et al., 2020) due to compatibility of nutritional requirements and biosynthetic capabilities of the microbial groups. This interaction is mediated by cross-feeding, exchange of metabolites (Ponomarova et al., 2017), different rates of nutrient transport and uptake by different species and strains, sensitivity to metabolic end products, response to killer toxins and production of quorum sensing molecules (Fleet, 2007). Although, these interactions can also govern the population between two microbial groups they can govern the population dynamics within the same microbial group. Reported interactions include the inhibition of LAB by ethanol produced by yeasts, and the death and autolysis of yeast cells releases vitamins and other bio-factors that stimulate the growth of bacteria, while

excessive growth of LAB and acetic acid bacteria increases the lactic and acetic acid concentration, respectively which inhibits the growth of yeasts (Fleet, 2007, Adesulu-Dahunsi et al., 2020). In particular yeasts are reported to produce primary metabolites during fermentation such as ethanol, amino acid metabolites and phenolic compounds (Tofalo et al., 2020).

Houngbédji et al. (2021) reported on the inhibition of *C. glabrata* by *Lactobacillus fermentum* and *Weissella confusa* due to the production of lactic and acetic acid produced by these species during fermentation. While Annan et al. (2002) found that the population of LAB in maize fermented with *S. cerevisiae* and *C. krusei* starter cultures ranged between 4.6×10^6 CFU/g – 6×10^6 CFU/g at time 0 h and between 1.2×10^9 CFU/g - 9.0×10^9 CFU/g at time 72 h, respectively. Thus, highlight the co-existence of both microbial groups. Furthermore, spontaneous fermentations where both microbial groups have been isolated have reported changes in amino acid content, organic acids concentration, volatile aroma compounds and vitamin content (Annan et al., 2002; Chaves-Lopez, et al., 2020; Chileshe et al., 2020; Halm et al., 1993). For example, traditional fermentation of Munkoyo yielded a higher concentration of the vitamins B1, B3 and B6. The bacterial population comprised of species from the genera *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Enterobacter*, *Klebsiella* and Acetobacter (Chileshe et al., 2020). Although the yeast population was not determined in this study, this product is associated with the proliferation of *S. cerevisiae* a yeast reported to synthesize folate (Hjortmo et al., 2005). Thus, the synthesis of the vitamins could be associated with the growth of both the bacterial and yeast population

In the study by Li et al. (2021), the co-inoculation of yeast with either LAB or acetic acid bacteria (AAB) or both yielded an increase in the lactic acid concentration in wheat dough of which the three-tier inoculation yielded a bread with a longer shelf life. The axenic yeast inoculation and co-inoculation of the yeast and LAB or AAB or both yielded between (33.46% and 21.76%), (21.95% and 12.15%) and (19.14% and 10.56%) proportion of the genes encoding the KEGG pathways nitrogen metabolism; alanine, aspartate and glutamate metabolism; and biosynthesis of amino acids, respectively. Research on microbial interactions in African fermented foods is mainly based on phenotypic factors whereas the use of high-throughput sequencing would provide detailed information and linkages to the diverse population in these fermentations.

4. NUTRITIONAL SIGNIFICANCE OF YEASTS IN AFRICAN MAIZE FERMENTED FOODS

Naturally fermented cereals make at least 80% of the total calorie consumption in Africa. However, the nutritional density of maize is weak and does not fulfil the nutritional requirements (McKevith, 2004). Therefore, commercially available maize products are fortified to meet the nutritional requirements of consumers (Palacios-Rojas et al., 2020). In Sub-Saharan African communities maize grain and/or its by–products are fermented prior to consumption. The fermented products are used either as complementary or weaning foods for infants and staples for adults (Chaves-López et al., 2020; Mensah et al., 1991).

4.1. *Macronutrient bioavailability*

Cereals including maize are reported to have low protein content and bioavailability thus resulting in essential amino acids deficiency. In non-fermented mawè, the amino acids glutamic acid and cysteine were present in high (130.7 mg) and low (1.3 mg) amount in 100 g (*dw*), respectively in the complex form. Whereas when the mawè was fermented with axenic yeast cultures, total free amino acids (TFAA) increase was 16.9%, 20% and 30.4% for *S. cerevisiae* strain 3, *P. kudriavzevii* strain 5 and *S. cerevisiae* strain 2 at 72 h, 48 h and 24 h, respectively. The *P. kudriavzevii* strain 2 yielded 47%, 45% and 79.4% TFAA increase at 24 h, 48 h and 72 h, respectively (Houngbédji et al., 2021). Ogunremi et al., 2015b reported that yeasts isolated from fermented cereal foods secrete protease, lipase and esterase enzymes which aid in the release of amino acids, flavour and aroma compounds during fermentation. Furthermore, *K. marxianus* possess β-glucosidase (Karim et al., 2020), β-D-galactosidase activity, inulinase and polygalacturonases (Fonseca et al., 2008) which are important in carbohydrate degradation.

4.2. Micronutrient bioavailability

Cereals possess high levels of the antinutrient phytate, with maize containing 0.89% (McKevith, 2004). Phytate is a polyanion that binds positively charged compounds or molecules thus is a strong chelating agent. Chelating function is attributed to the strong mineral complexes established between phytate and divalent ions such as iron, calcium, magnesium and zinc. The presence of phytate in foods limits the bioaccessibility and availability of micronutrients, thus it is recognized as an antinutritional factor (Gabaza et al., 2017). In maize, minerals are mostly present in the germ and are found in low quantities when compared to other cereals. Thus, further limitation in quantity of bioaccessible and bioavailable minerals in

cereals thus contributing to micronutrient deficiencies in communities where cereals are staple foods.

It's been reported that fermentation has a beneficial effect in nutritional quality of fermented foods. This is not only attributed to yeast growth during fermentation but also to enzymatic activity of the yeasts. Yeasts are known to secrete a myriad of extracellular enzymes such as glucosidases, lipases, proteases and glucanases (Gamero et al., 2016). One particular enzyme is phytase (Olstorpe et al., 2009), which hydrolyzes phytate to lower inositol phosphates of different isomeric forms (Gabaza et al., 2017). Phytase activity and the genes encoding the enzyme have been identified in a number of yeasts isolated from cereal fermentations (Greppi et al., 2015; Hellström et al., 2010; Nuobariene et al., 2011). The enzyme can either be secreted intracellularly, periplasmatic, extracellularly to the culture medium or bound to the yeast cell wall (Greppi et al., 2015). Expression of the *PHYPk* gene and subsequent enzyme activity from *P. kudriavzevii* strains isolated from cereal fermentations varies. Only one strain (G6) isolated from *gowé* showed highest gene expression between 250 and 300 relative mRNA expression. The rest of the strains isolated from *gowé* and *mawè* showed less than 120 relative mRNA expression. The lowest recorded expression was at 0.8 relative mRNA expression. Enzymatic activity followed a similar pattern such that strain G6 and G5 had between 35 and 40 mU/mL extracellular enzyme activity at 2 h and 8 h, respectively. Whereas strains (M31 and M30) isolated from *mawè*, showed similar enzyme activity at 2 h although with mRNA expression at 9-10 and 100-120 relative mRNA expression, respectively at this period (Greppi et al., 2015).

The phytate content of maize prepared togwa was found the highest at 4.7 µmol/g compared to sorghum (2.6 µmol/g) and cassava (0.4 µmol/g) prepared togwa (Hellström et al., 2010). At this phytate content it was found that maize had the largest amount of total iron and soluble iron content compared to sorghum and cassava prepared togwa. However, this resulted in 5.0% of soluble iron. In order to have a positive effect on iron absorption, the molar ratio (phytate:iron) should be 1:1 and below 0.4:1. In this study by Hellstrom *et al*. (2010), the phytate and iron ratio for the maize togwa was 3.1:1 thus phytate degradation was not sufficient. On the other hand, the zinc content of the prepared togwa products was mainly accounted for in the sorghum based preparation.

Phytase activity is dependent on pH with cereal endogenous phytases having a pH optima between pH 4.5 and 5.0. During cereal fermentation, the pH optima is between pH 3 and 6 (Table 1). Yeast phytase activity is affected by concentration of external free inorganic

phosphate were high inorganic phosphate represses phytase production by the yeast while low inorganic phosphate enhances phytase expression (Hellström et al., 2010). Cell growth was found to not have an effect of phytate degradation i.e. phytase activity (Hellström et al., 2010). Of all the isolates found in togwa fermentation, the species *I. orientalis* and *H. guilliermondii* were the most prominent phytase producers (Hellström et al., 2010).

4.3. Biosynthesis of folic acid

Vitamin B9 (folic acid) is an essential water soluble fully oxidized monoglutamate form of folate (Saini et al., 2016). The vitamin plays an important role in cellular metabolism where its derivatives act as coenzymes C1 transfer reactions in the synthesis of amino acids, nucleotide (purines and pyrimidines) biosynthesis and, oxidation and reduction of one carbon units required for normal cell division and growth (Hjortmo et al., 2005; Revuelta et al., 2018; Saini et al., 2016). Folate derivatives i.e. dihydrofolate, tetrahydrofolate (THF), L-methyl folate are the main participants in biological activities. Folate deficiency poses a risk factor in chronic diseases and developmental disorders e.g. autism, Alzheimer's disease, senile dementia, neural tube defects (Saini et al., 2016). Fungi synthesize the vitamin *de novo* while mammals obtain folate through diet (Goncerzewicz and Misiewicz, 2015). Folate derivatives (THF; 5-methyl-THF; 5,10-methylene-THF; 5,10-methenyl-THF and 10-formyl-THF) are interconverted by accepting or donating C1 groups during the reactions in the biosynthesis of folate (Figure 1) (Revuelta et al., 2018).

The Recommended Dietary Allowance (RDA) of folate is calculated in micrograms of dietary folate equivalents (DFEs) based on the bioavailability of the different forms of folate when 50% natural folate and 85% folic acid is bioavailable when taken with food. Thus, 1 µg DFE is equivalent to 1 µg food folate and 0.5 µg folic acids from dietary supplements (Saini et al., 2016). As summarized by Saini et al. (2016) legumes contain the highest folate content between 399 and 658 µg/100 g in comparison with food groups such as cereals, fruits, vegetables, spices and herbs, and dairy and egg products. Furthermore, dietary intake of folate is also associated with the consumption of fermented cereals (Greppi et al., 2017). Saini et al. (2016), reported cereals to contain between 8 and 231 µg/100 g of folate content.

Fermentation is known to enhance vitamin content of food through the activity of the microbial population and the biochemical reactions taking place (Blandino et al., 2003). Bationo et al. (2020) found that the folate content of the raw material was influenced by initial processing of

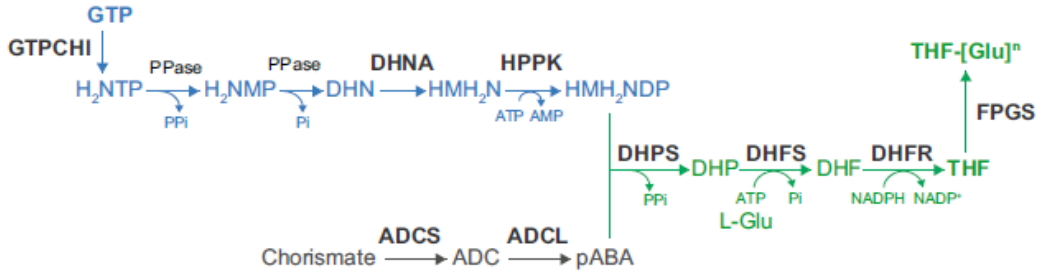

Figure 1: Folate biosynthesis pathway (Revuelta et al., 2018)

Metabolites: H_2NTP = 7,8-dihydroneopterin triphosphate; H_2NMP = 7,8-dihydroneopterin monophosphate; DHN = 7,8-dihydroneopterin; HMH_2N = 6-hydroxymethyl-7,8-dihydropterin; HMH_2NDP = 6-hydroxymethyl-7,8-dihydroneopterin diphosphate; ADC, 4-amino-4-deoxychorismate; DHP = 7,8-dihydropteroate; DHF = 7,8-dihydrofolate; THF = tetrahydrofolate.

Enzymes: GTPCHI = GTP cyclohydrolase I; PPase = phosphatase; DHNA = dihydroneopterin aldolase; HPPK = 2-amino-4-hydroxy-6-hydroxymethyldihydropterin pirophosphokinase; ADCS = aminodeoxychorismate synthase; ADCL = 4-amino-4-deoxychorismate lyase; DHPS = dihydropteroate synthase; DHFS = dihydrofolate synthase; DHFR = dihydrofolate reductase; FPGS = folylpolyglutamate synthase.

the maize grain. Debranning and degerming resulted in losses between 23% and 66% were whole grain maize had an initial folate content between 13.8 and 24.3 µg/100 g DM (dry matter) and after debranning folate content decreased to between 5.0 and 12.8 µg/100 g DM. Only fermented foods (*akassa* and *doncounou*) prepared from the raw material yielded 78% and 7% folate gain from producer 1 and 2, respectively. The foods from two other producers yielded folate losses between 10% and 22% for *akassa* and *doncounou*, respectively. These losses further hampered on bioaccessible folate which was low at 0.4-3.2 µg/100 g FW (fresh weight) for all the fermented foods. Folate bioaccessibility was only high for *doncounou* (57%-67%) while for *akassa* it was low at 24%-29% (Table 2). The microbial population of these foods was not determined.

The fermentative yeast *Saccharomyces cerevisiae* is recognized as a producer of folate (Kariluoto et al., 2006; Patring et al., 2006; Pietercelie et al., 2011; Walkey et al., 2015). The genetic make-up *of S. cerevisiae* favours the synthesis of folate due to the possession of the genes (*FOL2, FOL3* and *DFR1*) coding for the enzymes (GTP-cyclohydrolase I, dihydrofolate synthase and dihydrofolate reductase), respectively (Goncerzewicz and Misiewicz, 2015). *S. cerevisiae* strains and related species were found to be the highest folate producers compared to other non-*Saccharomyces* yeasts screened (Hjortmo et al., 2005). Furthermore, folate synthesis was found to be species, cultivation conditions and growth phase dependent when *S. cerevisiae* and non-*Saccharomyces* species were investigated in laboratory media and togwa (fermented maize with sorghum or millet) (Hjortmo et al., 2005; Hjortmo et al., 2008a, 2008b). *S. cerevisiae* has a folate content ranging from 1-4 mg/100 g pressed yeast (Witthöft et al., 1999) with the folate derivatives: (6S)-5,6,7,8-tetrahydrofolate sodium salt (H_4folate), (6S)-5-HCO-5,6,7,8-tetrahydrofolate sodium salt (5-HCO-H_4folate) and (6S)—CH_3-5,6,7,8-tetrahydrofolate sodium salt (5-CH_3-H_4folate) identified in the fermentation biomass (Hjortmo et al., 2008b). The dominant folate forms in the yeast biomass are 5-methylated tetrahydrofolate (5-CH_3-H_4folate) and polyglutamylated tetrahydrofolate (H_4folate), with (5-CH_3-H_4folate) constituting 65% of the total folate content in both *S. cerevisiae* and non-*Saccharomyces* strains studied (Hjortmo et al., 2005).

Table 2: Effect of fermentation on total folate content, bioaccessible folate and folate bioaccessibility on maize fermented products (Bationo et al., 2020)

Maize food product and type	**Producer**	**Total folate content (µg/100 g *fw*)**		**Bioaccessible folate (µg/100 g *fw*)**	**Folate bioaccessibility (%)**
Gelatinized dough		**Raw material**	**Fermented food**		
Akassa	P1	13.8	3.1	0.9	29.1
	P2	16.8	1.8	0.4	23.6
	P3	21.2	2.7	0.7	26.4
Doncounou	P1	15.7	4.7	3.2	66.9
	P2	14.8	4.8	2.8	57.4
	P3	13.8	3.6	2.2	62.4

Folate bioaccessibility (%) = Bioaccessible folate/ Total folate x 100

fw = fresh weight; P1-3 = producers of gelatinized dough

5. FERMENTATION OF MAIZE FOODS AND ASSOCIATED HEALTH PROMOTING PROPERTIES

5.1. Probiotic effect

According to the FAO/WHO, probiotics are live microorganisms that when administered in adequate amounts confer a health benefit on the host. The use of probiotics is to confer health related properties such as pathogen interference, exclusion or antagonism, immune modulation, anticarcinogenic and antimutagenic activities, alleviation of lactose intolerance symptoms, reduction in serum cholesterol levels, reduction of diarrhoea, prevention of bacterial vaginosis and urinary tract infection, maintenance of mucosal integrity and improved periodontal health (Franz et al., 2014). Published guidelines by the FAO/WHO for probiotic classification are the accurate identification of the strain to be used, verification of its safety, non-toxic status and be able to provide a physiological and health benefit in randomized clinical trials.

Saccharomyces boulardii is a commercially available probiotic yeast (Kelesidis and Pothoulakis, 2012) and is recommended for the prevention and treatment of human gastrointestinal diseases (Agarbati et al., 2020). Although bacteria are mostly reported with probiotic properties, yeast species from fermented and non-fermented foods also possess properties to be considered as probiotics (Agarbati et al., 2020; Greppi et al., 2017). Main properties for consideration are the ability to survive in the gastrointestinal tract i.e. growth at 37°C, tolerate acidic pH and bile salts; adhere to and persist in the mucosal and epithelial surfaces, possess antimicrobial activity against human pathogens. Screening by Agarbati et al. (2020) revealed that *Lachancea thermotolerans*, *Metschnikowia ziziphicola*, *S. cerevisiae* and *Torulaspora delbrueckii* yeasts strains compared well with CODEX on the parameters tested for probiotic potential. Whereas the yeasts *P. kluyveri*, *P. kudriavzevii* and *C. tropicalis* isolated from fermented cereals showed 100% and 15% survival at pH 2, respectively (Ogunremi et al., 2015a).

5.2. Fermentation induced changes in the phytochemical profile compounds and related bioactive properties

Cereals consist of various classes of phytochemicals in which phenolic compounds such as phenolic acids, tannins, and flavonoids are dominant (Wang et al., 2014). Phenolic compounds are chemical substances that consist of aromatic rings with one or more hydroxyl groups in their structures (Siyuan et al., 2018). These compounds are involved in the modulation of cellular oxidative status and subsequent inhibition of oxidative damage of biomolecules such

as membrane lipids, proteins, and DNA (Wang et al., 2014). Therefore, reducing the incidence of cardiovascular diseases, type 2 diabetes, and oxidation induced cancers.

Cereal grain phytochemicals such as phenolic acids and flavonoids are mainly found in the outer parts of the grain (Bondia-Pons et al., 2009). The composition of phytochemicals differs according to the cereal grain variety (Siyuan et al., 2018) and compounds may accumulate in different tissues and cells (Boz, 2015). Some phenolic acids are present in the starchy endosperm of the kernel. For example, in unprocessed rye bran, phenolic acids form part of the dietary fibre complex of the bran, cross-linking with polysaccharides and thus strengthening the insoluble cell wall structures (Faulds and Williamson, 1999). Phenolic acids such as syringic acid, vannilic acid, caffeic acids, ferulic and *p*-courmaric have been profiled in maize (Siyuan et al., 2018) and 87% of these are present in bound form. Similarly, about 58%, 71% and 90% of phenolic compounds in oats, rice and wheat are present in bound form (Sandhu et al., 2017). These compounds are linked by ester bonds to cell wall structural components such as proteins, lignin and cellulose (Siyuan et al., 2018). Particularly, ferulic acid is mostly ester bound to heteroxylans (Andreasen et al., 2000), making it unavailable for absorption during digestion.

Fermentation is used as a tool to promote the release and/or to enhance the accessibility of bound phenolic compounds in cereal grains (Wang et al., 2014) affecting the amount and the profile of phytochemicals in the grains. Fermentation metabolic pathways are involved in the production of vital bioactive molecules that include phenolic compounds (Adebo and Gabiela-Medina-Meza, 2020). How these phytochemicals are altered by microbial activity and metabolism is an area that requires attention when investigating the health promoting properties of fermented foods.

Extracellular enzymes such as phytases, amylases, esterases and lipases (Chaves-López et al., 2020) secreted during fermentation by yeasts may produce simpler forms of complex substrate through hydrolysis and transformation of some biomolecules into bioactive forms (Rai et al., 2019). *S. cerevisiae* strain has been reported to produce β-glucosidases and feruloyl esterases which are responsible for the production of free phenolics from conjugated and bound phenolics (Moore et al., 2007; Rai et al., 2019). In a study by Oladeji et al. (2017), fermentation reduced the total phenolic contents (TPC), total flavonoid content (TFC) and antioxidant activities of ogi after 96 h. The decrease could be attributed to phenolics being metabolized by microorganisms in the substrate. For instance, *S. cerevisiae* has been shown to produce an

active anthocyanin-β-glucosidase that can significantly reduce the presence of anthocyanin in wines. Furthermore, phenolic compounds could be adsorbed into the yeast cell wall through their interaction with exterior mannoproteins situated on the yeast cell wall (Zhang et al., 2021).

The effect of yeast metabolism on the phenolic profile of maize fermented products has not been fully exploited and requires more attention. However, in other grain products, fermentation has contributed to an increase in phenolic contents. For example, spontaneous fermentation of finger millet flours for 96 h showed an increase in TPC, TFC and antioxidant activity (Mutshinyani et al., 2020). In spontaneous fermentation, yeasts coexist with LAB therefore effects noted could be attributed to both organisms. Koistinen et al. (2016) reported that rye bran bioprocessing by sourdough fermentation process or enzymatic treatment caused partial degradation of the grain cell walls, specifically the activity of ferulic acid esterase released phenolic compounds from the fibre matrix of the rye bran, thus improving their bioavailability. In whole-wheat pizza dough, fermentation significantly increased the contents of soluble free ferulic acids of Trego and Lakin wheat varieties (Moore et al., 2007). The improved presence of phenolics can be attributed to the ability of microorganisms (strain-dependent) to convert phenolic compounds, such as the hydrolytic cleavage of ester-linked sugars from flavonoids and ferulic acid, and decarboxylation of hydroxybenzoic and hydroxycinnamic acids (Gänzle, 2014). Decarboxylation of phenolics by yeast cells may occur through the actions of ferulic acid decarboxylase (FDC1) which functions in the presence of a cofactor (modified flavin mononucleotide (FMN)) produced by phenylacrylic acid decarboxylase (PAD1) (Dzialo et al., 2017).

5.3. Fermentation derived peptides and associated bioactive properties

Dominant yeasts during fermentation may act upon protein from the substrate to produce bioactive peptides via proteolytic activity (Rai et al., 2019). Carboxypeptidases and aminopeptidases are produced by yeasts in accordance with their amino acid auxotrophy (Mirzaei et al., 2021). Carboxypeptidases can hydrolyze polypeptides to single amino acids while aminopeptidases can cleave the N-terminal of proteins into single amino acids, dipeptides or tripeptides (Mótyán et al., 2013), hence, producing a diverse range of bioactive peptides. Bioactive peptides usually consist of 2-20 amino acid fragments that possess various biological activities after their release from the parent proteins (Mirzaei et al., 2021).

A wealth of data exists on bioactive peptides produced as a result of LAB during cereal fermentation with limited information on yeast fermentation derived peptides. However,

unfermented maize products have been shown to possess bioactive peptides produced via enzymatic hydrolysis of kernels and sub-products (Díaz-Gómez et al., 2017). Alcalase-treated zein hydrolysate produced bioactive peptides Tyr-Ala and Leu-Met-Cys-His with $ABTS^{\bullet+}$, DPPH and $O_2^{\bullet-}$ radical scavenging activity (Tang et al., 2010). Fermentation of defatted adlay (*Coix lachryma-jobi* L.) with *S. cerevisiae* (active yeast) was found to contribute to an increase in peptides with a low molecular weight (MW) of 0.18–0.5 kDa and <0.18 kDa while 2–15 kDa gradually decreased with fermentation time up to 60 h (Xu et al., 2021). The fermentation of wheat bran with *S. cerevisiae* exhibited an increase in 0.18-3 kDa peptides and decrease in 3-15 kDa peptides after 24 h (Zhao et al., 2017). Further research into characterization, sequencing and bioactivity determination of these produced peptides is required.

The biological activities of peptides such as the antihypertensive, antioxidant, antimicrobial, immunomodulatory and cholesterol reducing properties are highly dependent on the size of peptides, the composition and sequence of amino acids (Rai et al., 2019). Antioxidant peptides are dominated by peptides of <3 kDa that consist of hydrophobic amino acid residues at the N and C terminal position (Mirzaei et al., 2021). Antioxidant activities of peptides are related to their radical scavenging properties by means of single electron transfer (SET) or hydrogen atom transfer (HAT) mechanisms (Mirzaei et al., 2021). Furthermore, the antioxidant activity of peptides is reported to occur due to ferric reducing activity, retardation of lipid oxidation, and upregulation of endogenous antioxidant enzymes in cells with oxidative stress (Mirzaei et al., 2021). Antihypertensive effects have also been reported in maize-derived peptides (<5 amino acid residues) due to the inhibitory action against the angiotensin-converting enzyme (ACE) (Díaz-Gómez et al., 2017). Antihypertensive peptides are characterized by the presence of aromatic and branched aliphatic amino acids in their sequence (Mirzaei et al., 2021). Small hydrophobic peptides of about 5-10 kDa are characteristic of antimicrobial compounds which exert their activity through interacting with negatively charged cell wall and plasma membrane components (Mirzaei et al., 2021). Antimicrobial peptides can easily cross the membrane of bacterial cells or increase the permeability of the cells through pore formation, hence, causing apoptosis of cells (Rizk et al., 2018).

6. CONCLUSION

Most of the reported nutritional and health benefits of fermented maize products are attributed to LAB proliferation. However, this appraisal also shows the role of various yeasts in the nutritional and health attributes of fermented cereal products. *Saccharomyces cerevisiae*,

Pichia kudriavzevii, Candida spp. and *Kluyveromyces* spp. are dominant in maize fermentations. These yeasts possess enzymes such as lipase, esterase and phytase. Phytase activity particularly, is an important factor in improving iron bioaccessibility/bioavailability in fermented maize. Furthermore, yeasts such as *S. cerevisiae* can synthesize and increase folate concentrations normally inadequate in decorticated and degermed maize based products. Yeasts are also responsible for the increase in the total free amino acids of fermented maize products. Although most probiotic properties of foods are ascribed to LAB, *P. kudriavzevii* and *I. orientalis* isolated from ogi were found to have probiotic properties. Limited information is available on the effects of yeasts on the phytochemical profile of various fermented maize products. However, it has been reported in other cereals that free ferulic acid content is improved after fermentation due to the ferulic acid decarboxylase synthesis by the yeasts. Further research is required in relation to phytochemicals and bioactive peptides produced as a result of yeast proliferation in maize thus signifying their importance in producing healthy products.

7. CONFLICT OF INTEREST

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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