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(Research Article)

The effects of *Sorghum bicolor* leaf stalk (SBLS) on the bacterial quality of Ogi produced from maize (*Zea mays*)

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Abstract

Ogi is a staple food in Nigeria and other West African countries given its affordability and easy preparation it is largely consumed by many people. Ogi is used as a weaning food and given to the elderly and convalescent. Studies have shown that nutrients are lost during the production of Ogi. Hence, Ogi is fortified with *Sorghum bicolor* leaves stalk (SBLS) to prevent malnutrition despite the antibacterial activities of the SBLS extracts against some bacteria strains but little is known on the activity of the SBLS fortified Ogi on the bacterial, physicochemical quality and proximate composition of Ogi. This study aims to investigate the effect of SBLS on the bacterial quality and other physicochemical properties of Ogi. SBLS was added to Ogi during the secondary fermentation for seven days. Samples were taken at time 0, 1, 3, 5 and 7 days. The samples were analysed to determine the total titratable acidity, pH, temperature, proximate composition and bacteria compositions in Ogi fortified with SBLS using unfortified Ogi samples as control. The fortification of Ogi with SBLS significantly reduced the percentage moisture, crude fat and ash content compared to the unfortified Ogi. There were significant increase in percentage crude fiber and crude protein of Ogi fortified with SBLS compared to unfortified ($p < 0.05$). This research showed that Ogi fortified with SBLS prevented the growth of *Bacillus subtilis* compared to unfortified Ogi with SBLS. The addition of SBLS inhibits the growth of *Bacillus subtilis* and thus affects the bacterial quality of Ogi.

Keywords: Ogi; *Sorghum bicolor*; Bacteria; Proximate; Temperature; Fermentation; Food

1. Introduction

'Ogi' is a fermented non-alcoholic starchy food and is a chief staple food regularly consumed in West Africa. It is a sour fine paste beverage which when cooked produces a thin semi solid porridge (Nwosu and Oyeka, 1998). It is also called 'Eko', 'Agidi', 'Akamu' and 'Koko' in Nigeria. The prepared Ogi could be very thick as in 'Agidi - a gel like food with 15% dry-matter concentration, Nago (1992) and Nago *et al*. (1998) or very soggy as in 'Koko' - a porridge having 10% dry-matter concentration. Thirdly is a semi-solid gelatinized mass referred to as 'eko elewe', 'ekokolobo' in south-western part of Nigeria. Ogi porridge has a smooth texture and a sour taste similar to that of yoghurt.

Ogi is commonly used as the first native meal given to babies at weaning to supplement breast milk, and it is an important morning cereal for pre-schoolers and adults. It is commonly used as a primary meal for convalescing patients since it is easily digestible. As a weaning food, it is primarily used by low-income families; it is estimated that over 25 million individuals use it 4-5 days per week (Banigo and Muller 1972). It is a popular and extensively consumed staple

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in many Nigerian homes due to its low cost, simplicity of preparation, and nutritional content (Adepoju *et al*., 2020). Anglani's (1998) review of the use of sorghum for human foods suggests that palm oil should be added to weaning gruel to boost its vitamin A content. Sorghum-based 'Ogi' is prepared similarly to maize-based 'Ogi' by steeping (soaking before wet milling), soaking in cold water for 3 to 5 days, and changing the water every day until it has a frothy top or smells of alcohol. Normally, by the fourth day, spoilage would have begun with off-odour majorly caused by *Brevibacteria spp* (Teniola and Odunfa, 2002).

Another preparation method is to soak in hot water (near to boiling) for 24 hours. The third procedure used in Benin involves cooking in boiling water for 10 minutes before steeping at room temperature for 12-48 hours (Nago *et al*., 1998). Other researchers (Nago *et al*., 1998; Akinrele, 2007) referred to these discrepancies as the Cold technique, the 'Fon' approach, and the 'Goun' procedure. Approximately 40% of total proteins are lost during the production of Ogi (Nago *et al*., 1998) using the 'Goun' process, but the digestibility of the residual proteins rose by 20%, with 50% of both macro-mineral and micro-mineral elements removed. Temperature and polysaccharide excretion during fermentation can significantly affect the ultimate viscosity (Abiodun *et al*., 2002).

Maize (Zea mays) is the world's most significant and extensively spread cereal, behind wheat and rice. It is utilised for three primary purposes: as a staple food crop for human consumption, as livestock feed, and as a raw material for a variety of industrial applications, including biofuel generation (Ibeawuchi *et al*., 2008). Maize porridge, or pap, is known as 'Ogi' and akamu in the Yoruba and Igbo tribes of Nigeria, respectively and akosa in Ghana (Adegbehingbe, 2013). 'Ogi' is a popular fermented meal consumed in several parts of Nigeria. 'Ogi' is one of the 30 Nigerian traditional fermented dishes mentioned by Iwoha and Eke (1996).

Ogi is made from a variety of cereal-based feedstocks, including maize (*Zea mays*), sorghum (*Sorghum sp*), guinea corn and millet (*Pennisetum typhoideum*) (Ohenhen and Ikenebomeh, 2007; Osungbaro, 2009; Adegbehingbe 2014; Adebukunola *et al*., 2015; Abioye and Aka, 2015). In general, 'Ogi' is mostly fermented in acidic conditions. According to Akinerele (1970), many bacteria are connected with the fermentation of pap ('Ogi'). They include Cephalosporium, Aspergillus, Penicillum, *Corynebacterium spp*., *Aerobacter cloacee*, and *Lactobacillus plantarum*, among others. Okafor (1987) isolated Pediococcus, Pentosaceus, and Candida species from the pap. Fields *et al*. (1981) discovered two strains of Lactobacillus (*Lactobacillus fermentum* and *Lactobacillus cellobiosus*) and Pediococcus in fermented maize meal mixed with water at 37°C.

Lactic acid bacteria (*Lactobacillus plantarum* and *Streptococcus lactis*) and yeasts (*Saccharomyces cerevisiae, Rhodotorula spp., Candida mycoderma*, and *Debaromyces hansenii*) also have been shown to be predominantly involved in fermentation of 'Ogi', playing important roles as aroma development, microbial stability and flavour enhancement (Aworh, 2008; Omemu *et al*., 2011). Lactic acid fermentation also helps to reduce anti-nutritional factors while enhancing nutrient density and antibacterial activity in the fermented food (Oyarekua, 2013). During 'Ogi' manufacture, nutrients such as protein and minerals are lost from the grains, reducing their nutritional value (Aminigo and Akingbala, 2004; Omemu, 2011; Ajanaku *et al*., 2010). 'Ogi' is typically produced on a small scale using traditional fermentation technolOgies. The microorganisms linked with the fermentation process are not stringently controlled neither is the succession arrangement and the influence of each of the microbial genera to the fermentation procedure obviously understood. *Sorghum bicolor* leaf stalk (SBLS) popularly known as 'poroporo' in the South-western part of Nigeria is known to possess antimicrobial activities against bacteria strains of bacteria like *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli*. The antimicrobial activity of SBLS is due to the presence of phytochemical compounds which include alkaloids, tannins, saponins, sterol and triterpenes (Salawu and Salimon, 2014). The addition of SBLS changes the colour with the addition of subtle flavour to Ogi.

SBLS is known to have the following health benefits: help boost digestive health, improve cardiovascular health, management of diabetes, prevents allergies in people with celiac disease, boost bone health and energy and decreases the risk of anaemia, boost brain function and helps in weight loss (Salawu and Salimon, 2014). The addition of SBLS in Ogi production has helped to improve the colour, taste and adds a subtle flavour to Ogi. Previous studies have shown that SBLS contained antimicrobial substances which are either bacteriostatic or bactericidal (Ali *et al*., 2020). However, little is known about the effect of the SBLS on the bacterial quality and other properties of Ogi. The present study is aims to investigate the effect of SBLS on the bacterial quality and other properties of Ogi.

2. Materials and methods

Four kilograms (4 kg) of dried clean, healthy and well sorted maize grains and 40 grams of dried powdered sorghum bicolor leaf stalk were purchased from Okusa market at Akungba-Akoko Ondo State Nigeria into a sterile ziplock bags. Two kilograms (2 kg) of the maize sample was weighed using a weighing balance (Mettler, UK). The maize grains were washed thoroughly with sterile distilled water and submerged in 6 litres of sterile distilled water to ferment for 3 days (72 h) in a clean sterilized covered bucket according to Adegbehingbe (2014). The fermented maize grains were wet-milled using a sterile electric blender (ATLAS, UK) and was sieved with sterilized muslin cloth with additional 4 litres of sterile distilled water. The sieved milled fermented maize was divided into two equal portions in a separate sterile containers. A 10 grams of powdered *Sorghum bicolor* leaf stalk (SBLS) was added and mixed thoroughly while the second portion remained without the addition of powdered SBLS (control) and allowed to ferment for seven days (1 week). The Water from the surface of the samples were collected aseptically and analysed at the day 0, 1, 3, 5 and day 7) for total titratable acidity (TTA), pH and temperature using the method described by Adegunwa *et al*., (2011); Adegbehingbe (2014). However, 1 gram each of the sediments from both samples were collected and serially diluted for bacterial analysis using nutrient agar (NA) (Oxoid, UK) and De Man-Rogosa-Sharpe agar (MRSA) (Oxoid, UK) for the isolation and enumeration of bacterial and lactic acid bacteria, respectively according to Adegunwa *et al*., (2011); Adegbehingbe (2014); and Adepoju *et al*., (2020). Samples were also taken at the final day (7th day) and analysed for the proximate composition according to Oyeleke (1984); Adegbehingbe (2014); and Adepoju *et al*., (2020). The bacteria isolated were identified according to Fawole and Osho, 2007; Adegbehingbe (2014); Bello (2022; 2023). Statistical analyses were performed using the program R 3.4.0 (http://www.rproject.org/). Data for TTA, pH, temperature and proximate compositions were analysed independently using one-way ANOVA (Statistical Procedures for Agricultural Research package (agricolae) with treatments and sampling time as factors. Tukey HSD multiple post-hoc tests were used to assess the significance of the differences between the means.

3. Results

The bacterial count increased progressively throughout secondary fermentation process both in the samples fortified with SBLS and in the samples without SBLS (figure1). But higher bacteria count were recorded in samples without fortification with SBLS compared to the fortified Ogi samples with SBLS throughout the sampling period, irrespective of the culture media used (nutrient agar and De Man-Rogosa-Sharpe agar (MRSA)). During the secondary fermentation of 'Ogi', eleven (11) bacterial species were isolated from 'Ogi' without *Sorghum bicolor* leaves stalk (SBLS) which includes *Lactobacillus plantarum, Corynebacterium striatum, Staphylococcus xylosus, Corynebacterium fermentum, Lactobacillus brevis, Lactobacillus fermentum, Leuconostoc mesenteroides, Pediococcus pentosaceus, Bacillus subtilis, Bacillus megaterium* and *Klebsiella oxytoca.* However, ten (10) bacterial species were isolated from 'Ogi' fortified with *Sorghum bicolor* leaves stalk (SBLS) which includes all the bacteria isolated from Ogi without fortification with SBLS except *Bacillus subtilis*. The predominant bacteria in both samples throughout the fermentation process were *Lactobacillus* species, *Leuconostoc* species and *Corynebacterium* species (table 1).

The total titratable acidity (TTA) of the fermentation process increased from 0.298 to 0.408 in sample A (without SBLS) while that of sample B (with SBLS) increased from 0.311 to 0.412 (figure 2). Hence, the pH in samples without SBLS and with SBLS reduced progressively from 3.37 to 2.99 and 3.29 to 2.88, respectively (figure 2). The temperature of samples without SBLS and with SBLS reduced progressively from 31.12°C to 28.05°C and 31.82°C to 28.36°C, respectively (figure 2). However, there was no significant different in TTA, pH and temperature between Ogi samples fortified with and without SBLS at the end of the secondary fermentation process for seven days ($p < 0.05$).

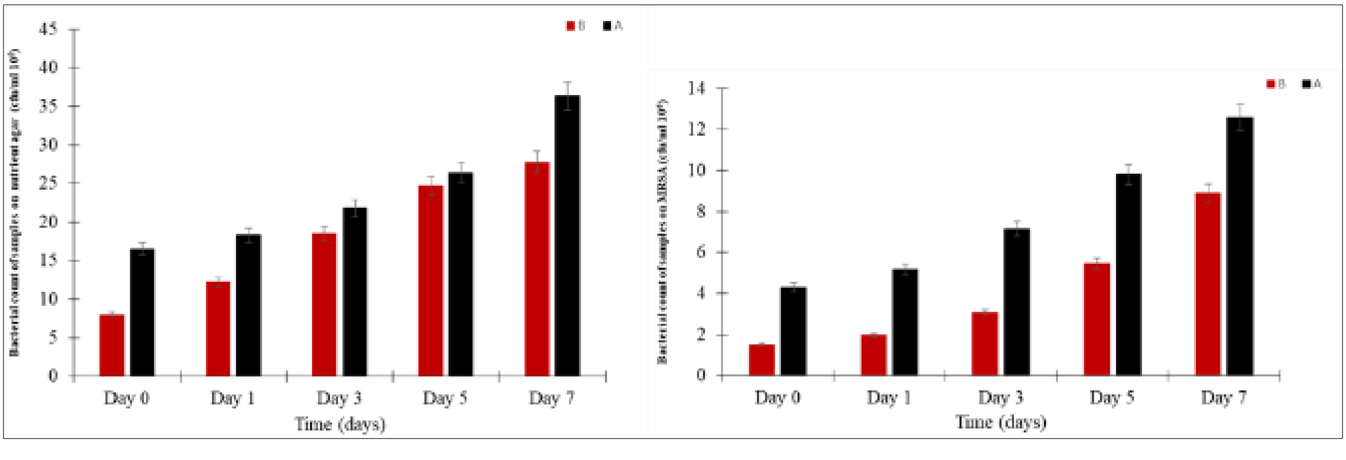

Figure 1 Bacterial colonies isolated from Ogi made from maize with and without *Sorghum bicolor* leaves stalk (SBLS) during secondary fermentation for seven days using Nutrient Agar and De Man-Rogosa-Sharpe agar (MRSA). Data represents the mean of triplicate sample while error bar represents the standard error of the mean. A= Sample without SBLS, B= Sample with SBLS

Additionally, the fortification of Ogi with SBLS significantly reduced the percentage moisture, crude fat and ash content compared to the unfortified Ogi (figure 3) ($p < 0.05$). However, there were significant increase in percentage crude fiber and crude protein of Ogi fortified with SBLS compared to unfortified ($p < 0.05$), while there was no significant difference in the percentage carbohydrate content of both the fortified and unfortified Ogi with SBLS (figure 3) ($p < 0.05$).

Table 1 The occurrence of bacterial species during secondary fermentation of 'Ogi' made from maize fortified with and without *Sorghum bicolor* leaf stalk (SBLS) (+ = present, - = absent)

Isolates	Day 0	Day 1	Day 3	Day 5	Day 7
Without *Sorghum bicolor* leaf stalk					
Lactobacillus plantarum	+	+	+	+	+
Corynebacterium striatum	+	+	+	+	+
Staphylococcus xylosus	+	+	-	-	-
Corynebacterium fermentum	+	+	+	+	+
Lactobacillus brevis	-	+	+	+	+
Lactobacillus fermentum	-	+	+	+	+
Leuconostoc mesenteroides	-	+	+	+	+
Pediococcus pentosaceus	+	+	+	-	-
Bacillus subtillis	+	+	+	-	-
Bacillus megaterium	+	+	-	-	-
Klebsiella oxytoca	-	-	+	+	+
With *Sorghum bicolor* leaf stalk					
Lactobacillus plantarum	+	+	+	+	+
Corynebacterium striatum	+	+	+	+	+
Staphylococcus xylosus	+	+	-	-	-
Corynebacterium fermentum	+	+	+	+	+
Lactobacillus brevis	-	+	+	+	+
Lactobacillus fermentum	-	+	+	+	+
Leuconostoc mesenteroides	-	+	+	+	+
Pediococcus pentosaceus	+	+	+	-	-
Bacillus megaterium	+	+	-	-	-
Klebsiella oxytoca	-	-	+	+	+

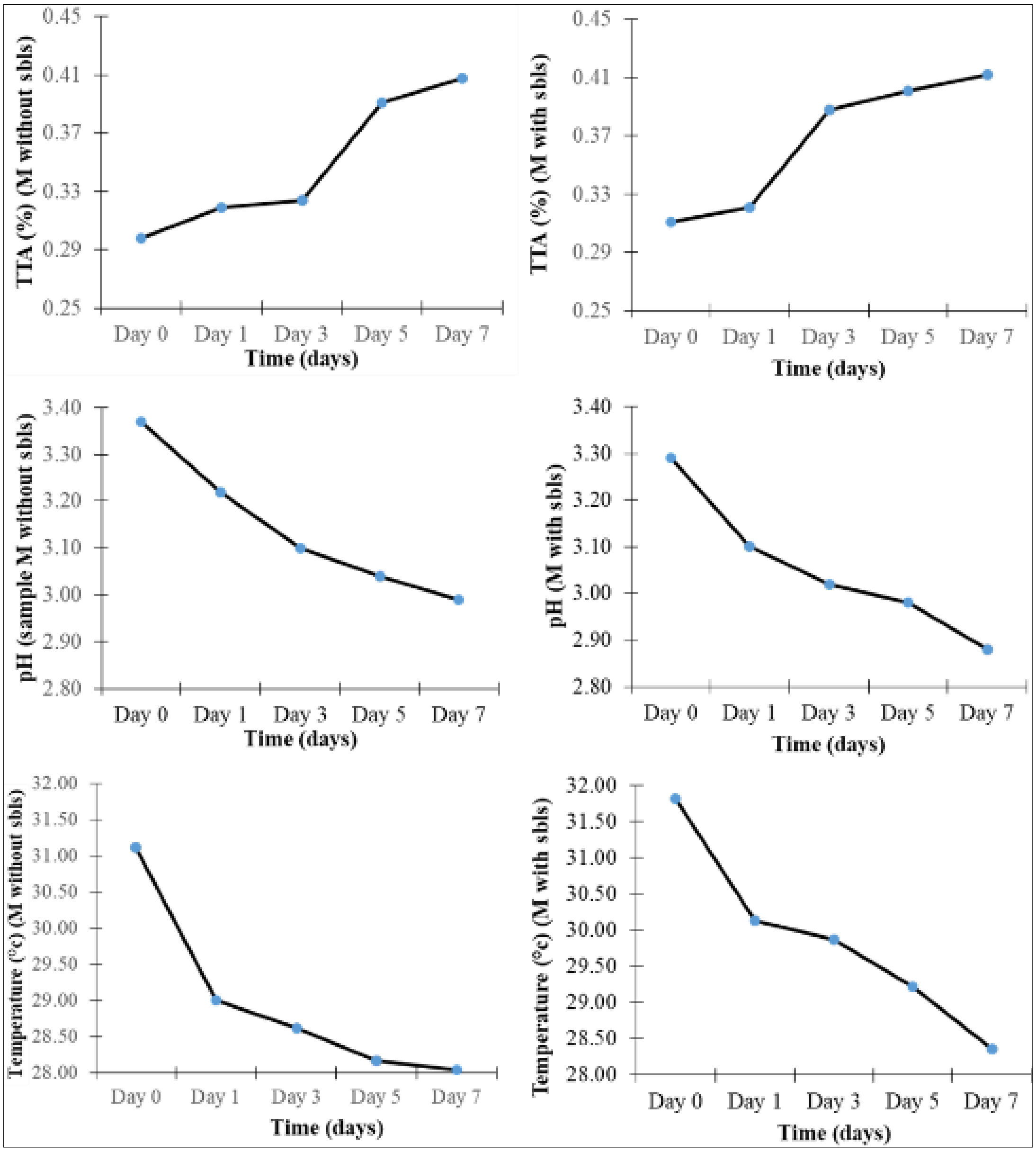

Figure 2 The Temperature, pH and Total Titratable Acidity (TTA) of Ogi made from maize with and without *Sorghum bicolor* leaves stalk (SBLS) during secondary fermentation of Ogi for seven days. Data represents the mean of triplicate sample

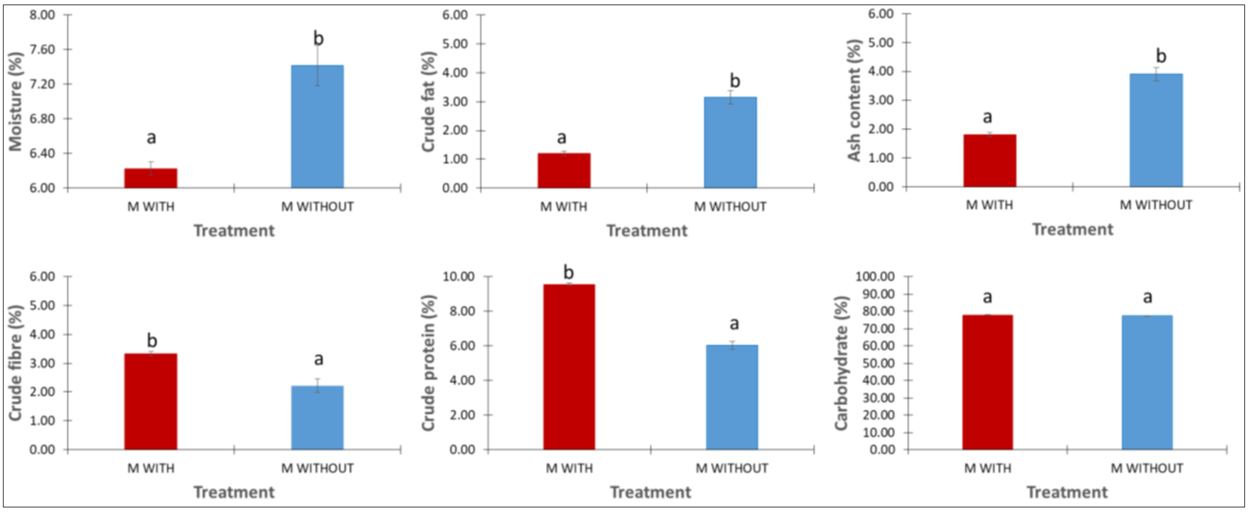

Figure 3 The proximate analysis of Ogi made from maize with and without *Sorghum bicolor* leaves after secondary fermentation for seven days. Data represents the mean of triplicate sample while error bar represents the standard error of mean, different alphabets represents significant different at $p < 0.05$

4. Discussion

It is a normal practice in some parts of Nigeria to add *Sorghum bicolor* leaf stalk (SBLS) to 'Ogi' made from maize purposely to improve the nutritional profile and to prevent malnutrition, but little is known on the impact of this fortification of Ogi with SBLS on the bacterial quality and proximate composition of Ogi. Bacteria isolated from both fortified and unfortified Ogi made from maize during present study include; *Bacillus megaterium, Bacillus subtillis, Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus brevis, Corynebacterium fermentum, Pediococcus acidilactici, and Leuconostoc mesenteroides*. The presence of these bacteria agrees with the previous studies by Fields *et al.* (1981), Aminigo and Akingbala (2004), Aworh (2008), Ojokoh *et al*. (2009), Omemu *et al*. (2011), Ajanaku *et al*. (2010) and Oyarekua (2013) who independently isolated similar bacteria in Ogi made from maize during secondary fermentation confirms that these bacteria isolates plays important roles in the development of aroma, stability and flavour enhancement in Ogi. The production of Lactic acid by *Lactobacillus fermentum, Lactobacillus plantarum* and *Lactobacillus brevis* during fermentation also plays important roles in reducing anti-nutritional factors in Ogi and increase both the nutrient density and antimicrobial activities in the fermented Ogi according to Oyarekua, (2013). The isolation of *Staphylococcus spp*. and *Klebsiella oxytoca* from both samples (with and without SBLS) are considered contaminants and could be as a result of cross contamination from the skin during laboratory activities or could be due to residue build-up in the milling machine, the environmental condition, the uncontrolled and spontaneous nature of fermentation, and the nature and nutritional contents of the substrate according to Vargas-Ramella *et al*. (2021).

The isolation of *Bacillus subtilis* in unfortified Ogi which is absent in the fortified Ogi indicates that SBLS contains anti-bacteria compound that inhibit *Bacillus subtilis.* This corroborates the previous studies by Salawu and Salimon (2014) and Ali *et al*. (2020) who independently confirmed the antibacterial activity of SBLS against bacteria like *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli.* According to Ali *et al.* (2020), the phytochemical screening carried out on *sorghum bicolor* leaves showed that it contains alkaloids, tannins, flavonoids, saponins, sterol, and triterpenes which are potential antibacterial agents. The results obtained from the present study also indicates the need for fortification of Ogi with SBLS as it selectively inhibits some bacteria and thus modify both the quantity and quality of bacterial that is present during the secondary fermentation of Ogi.

The increased in total titratable acidity coupled with decreased pH indicates that acids (e.g. ascorbic acid, butyric acid and lactic acid) concentration or production increased during the secondary fermentation of Ogi, irrespective of the fortification with of Ogi SBLS. This observation agrees with the previous studies by Ojokoh *et al*. (2009), Mal *et al*. (2010), Wakil and Daodu (2011) Wakil and Kazeem (2012) and Onovo (2014) during the secondary fermentation of maize in the production of Ogi. The lack of significant difference in both the TTA and pH values of the fortified and unfortified Ogi indicates that fortification of Ogi with SBLS has no impact on both the TTA and pH of Ogi during the secondary fermentation process. However, the decreased in the temperature from 31^{o}C to 28^{o}C observed during the secondary fermentation of Ogi irrespective of the fortification with or without SBLS agrees with previous studies by Ojokoh *et al*. (2009), Wakil and Daodu (2011) and Adegbehingbe (2014).

The fortification of Ogi with SBLS reduced its percentage moisture content, fat content and ash content but increased the crude protein and fiber. The reduction in the percentage crude fat contents of the fortified Ogi with SBLS could enhance their usefulness as weaning foods because food with high fat could be difficult to mix, which might also require antioxidants for preventing oxidative rancidity of the food according to Olukoya *et al*. (2017). This effect can be beneficial for weight loss and overall human health compared to unfortified Ogi with SBLS. The decrease in percentage ash content in Ogi fortified with SBLS compared to unfortified Ogi might be due to increased leaching of soluble inorganic salts into the processing water during fermentation by the SBLS. The results obtained in the present study corroborates previous studies by Ajanaku *et al*. (2010) and Adegbehingbe *et al*. (2014) who obtained a reduced ash content from complemented compared to non-complemented Ogi during fermentation from their independent studies. The reduction in ash content in fortified Ogi with SBLS is highly valuable as it eases digestion and shows a decrease the indigestible constituents of fortified Ogi with SBLS compared to unfortified Ogi with SBLS.

The increased protein and fiber content of fortified Ogi with SBLS compared to unfortified Ogi might be due to the increase in cell biomass, availability of nutrients from SBLS stimulating the growth of fermenting microorganisms in forms of single cell protein and ability of microorganisms to secrete protease coupled with suggestion that SBLS contains protein. This observation corroborates previous studies by Adegunwa *et al*. (2011) and Bolaji *et al*. (2015) who independently reported an increase in protein and fibre content of various fermented cereal blends. The increase in the protein content is highly beneficial as previous study by Salawu and Salimon (2014) shown that SBLS has a lot of health benefits such as prevention of allergies in people with celiac disease, boost bone health and energy, decreases the risk of anaemia, boost brain function and helps in weight loss. While the increase in crude fibre is important as it aids digestions, bowel movement and prevents colon cancer. The presence of high percentage of carbohydrate in both fortified and unfortified Ogi with SBLS agrees with previous studies by Olajuwon *et al*. (2022) which shows that Ogi contains about 77 % of carbohydrate which is a great source for healthy carbohydrate content and is a good source of energy that is beneficial to all age groups.

Based on the result obtained it is clear that addition of SBLS leaves to maize during production of Ogi, affect bacteria quality of Ogi as compared to unfortified Ogi. It also increases the crude protein and crude fiber, reduces the moisture content, ash content and crude fat composition of Ogi. This information can be considered when preparing 'Ogi' as a weaning formulae as *Sorghum bicolor* leaf stalks (ewe poroporo) adds to the nutritional profile of Ogi.

5. Conclusion

The fortification of maize with sbls during production of ogi, affect bacteria quality of fortified ogi as compared to unfortified ogi. The fortified ogi with sbls increases the crude protein and crude fiber but reduces the crude fat composition of ogi which is of great health benefits to people of all age. This information form the present study is of high benefit in the preparation of 'ogi' for both young and old as sbls adds to the nutritional contents of ogi.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Author's contributions

Authors contributed equally to the finance, designing of the experiment, laboratory analysis, statistical analysis and writing of the manuscript.

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