Multiple starter cultures fermentation of salted *soybean-daddawa*- a West African condiment

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Abstract

Study had suggested that multiple starter cultures fermentation of *soybean-daddawa* holds great promise for its industrialization but there is no report on effect of salt (NaCl) inclusion on the optimized process. The present work investigated the influence of NaCl inclusion on multiple starter cultures fermentation of *soybean-daddawa*. Salted (1%) and salt-free seeds of soybean were fermented into *soybean-daddawa* using multiple culture systems of *Bacillus subtilis* LB3, *Staphylococcus xylosus* SAU3 and *Leuconostoc mesenteroides* ssp *cremoris* LAB5. The different fermentation sets were assessed for protease and α-amylase enzyme activities (U/ml), Free Amino Acids (FAA) and Total Soluble Sugars (TSS) (mg/g), pH and Titratable acidity (TTA) (mg/g lactic acid). Sensory evaluation of the products was also carried out. In fermenting salted seeds, protease and α-amylase enzyme activities increased from 396 – 997, and 81- 209 respectively while the corresponding values for the salt-free samples were 414 – 914, 76 – 184. FAA contents of fermented salted and salt-free seeds were 30.3 - 36.7 and 30.6 – 38.1 respectively and the values for TSS were 88.8 – 95.3, 85.6 – 106 respectively. At the end of 65 h fermentation, no significant difference ($p > 0.05$) existed in the pH of all the fermented *soybean-daddawa*. Sample obtained using mixed culture of *Staphylococcus xylosus* SAU3 and *Leuconostoc mesenteroides* ssp *cremoris* LAB5 resulted in the highest TTA value of 4.3 mg/g lactic acid in the fermented salted seeds.. All the fermented seeds were organoleptically acceptable to the *soybean-daddawa* consumers but the panellist demonstrated highest preference for salted *soybean-daddawa* fermented with the 'cocktail' culture of *Bacillus subtilis* LB3, *Staphylococcus xylosus* SAU3 and *Leuconostoc mesenteroides* ssp *cremoris* LAB5. Results of the present study suggest that inclusion of NaCl (1%) has the capability of consolidating on the potentials of multiple starter culture fermentation of *soybean-daddawa*. This holds a great promise for subsequent industrialization of *soybean-daddawa* production.

Keywords: Industrialization; optimization; starter Culture; *soybean-daddawa*; table salt

1. Introduction

The dramatic increase for food secured Africa, exacerbated by the sprawling African population, is one of the biggest challenge most African countries will need to confront between now and 2050 (Fawole and Kolapo, 2022); as both protein calorie malnutrition and macro and micronutrients malnutrition are common characteristics of many African populations (Kolapo, 2011). In rural population across Africa and Asia, traditional fermented foods of legumes and oilseeds origin are being used to meet protein requirements of most poor households (Adedeji et al., 2017). In West Africa, *Bacillus* fermented legumes such as *Daddawa, Ogiri* (Adedeji et al*.,* 2017), Soumballa (Ouoba et al., 2003), *Soybean-daddawa* (Popoola and Akueshi, 1985, Omafuvbe et al*.,* 2000; Kolapo et al., 2007a, b; Adamu et al., 2019; Tersoo-Abiem et al., 2021; Ahmed et al., 2023) are very popular as condiments in soup and sources of dietary protein.

Industrialization of the production process of most African indigenous food products is far from being attained as production of *Soybean-daddawa* is largely done by spontaneous fermentation. However, it is worthy to note that in the last four decades, plethora of studies on *Soybean-daddawa* focused on processing, nutritional evaluation, process optimization, storage and preservation (Popoola and Akueshi, 1985, 1986; Kolapo and Sanni, 2006, 2007; Kolapo and Popoola, 2019; Kolapo and Popoola 2020 a, b; Kolapo et al., 2020; Karamba and Abdullahi, 2022). In this connection, previous optimization studies have reported on the use of single cultures of *Bacillus subtilis*, *B. licheniformis, B. pumilus* or in combinations (Suberu and Akinyanju, 1996; Omafuvbe et al., 2002). Afolabi and Abdulkadir (2016) improved on the restrictive use of *Bacillus* starter by incorporating *Leuconostoc mesenteroides* to *Bacillus subtilis* fermentation of *Soybean-daddawa.* While acknowledging that these optimization attempts achieved good quality end products, it is important to add that the traditional aroma of *soybean daddawa* was not perfectly replicated in these attempts. However, recent reports have documented the use of *Bacillus subtilis* LB3, *Staphylococcus xylosus* SAU3 and *Leuconostoc mesenteroides* ssp *cremoris* LAB5 for controlled fermentation of *soybean-daddawa*, in which the sensory attributes of naturally fermented *soybean daddawa* was nearly replicated in a controlled setting (Kolapo et al., 2019 a, b . Though, these recent optimization studies appears to hold great promise for *Soybean-daddawa* industrialization, there is no report on effect of table salt (NaCl) inclusion on this optimization process.

From the ancient time, sodium chloride has been widely used in food processing which affects sensory characteristics and safety and its presence is frequently essential for the proper preservation of the products (Bautista-gallego et al., 2013). Currently, the functional properties of salt in food processing and food production go well beyond taste as it plays different technological roles in food production. Besides flavour, it has an important role also in safety and on textural properties. It also influences the growth of bacteria involved in fermentation processes (Inguglia et al., 2019). In this regard, previous studies showed that 1% salt improved the organoleptic attributes of traditional fermented *soybean-daddawa* (Omafuvbe, 1994), and could be a practical way of extending the shelf-life of the product without affecting other functional and organoleptic properties (Popoola et al., 2007).

Though sodium salt is a pivotal ingredient in traditional fermented foods, but its excessive consumption adversely affects human health, product quality, and production efficiency. Therefore, reducing sodium salt content in traditional fermented foods and developing low-sodium fermented foods have attracted increasing attention (Lin et al., 2021). To this end, the World Health Organization (WHO) recommends that adults consume less than 5 g of salt per day (WHO, 2016). In a proactive response, several governments from countries around the world have adopted national salt reduction strategies, which range from legal obligations, such as the limit of salt content in bread, to intended actions involving the food industry, mainly regarding reformulating of food products (Trieu et al., 2015). In some European Union (EU) countries for example, < 2 %, ≤ 3.5 %, and < 4 % salt are permitted in bread, sausage and concentrated tomato puree paste respectively (Elias et al., 2019).

Report has shown that the addition of 1% salt improved the organoleptic quality of *soybean-daddawa* produced by monoculture of *B. subtilis* SDA culture (Omafuvbe, 2006). On the understanding that previous studies had suggested that multiple starter cultures fermentation of *soybean-daddawa* holds great promise for its industrialization and that there is no report on effect of salt inclusion on the multiple starter culture optimized process, the present work therefore investigated the influence of NaCl (1%) inclusion on multiple starter cultures fermentation of *soybean-daddawa*.

2. Methodology and methods

2.1 Starter Cultures

Previously typed, screened and selected cultures of *Bacillus subtilis* LB3, *Staphylococcus xylosus* SAU3 and *Leuconostoc mesenteroides* ssp *cremoris* LAB5 (Kolapo et al., 2019a) were obtained from the culture collection center of Biological Sciences Department, Augustine University, Ilara-Epe, Lagos State, Nigeria. Stock cultures of the first and second microorganisms were stored at - 40̊ C in nutrient broth (Difco, Michigan, USA) containing 20% (v/v) glycerol while MRS broth (Difco, Michigan, USA) was the media of choice for storage of the third microorganism. The cultures were reactivated by inoculating in relevant broth at 33 °C for 24 to 48 h.

2.2 Preparation of Soybean-Daddawa by Starter Culture Fermentation

Eight batches of *Soybean-daddawa* were produced in a controlled setting replacing the rudimentary equipment used in the traditional method with glassware as described earlier by Kolapo et al. (2019a) except that both dehulled and cooked salted (1%) and salt-free soybean seeds were used. Starter cultures selected on the basis of their previously described technological characteristics were introduced into the fermentation medium at the onset of fermentation. They were used as multiple cultures as shown in table 1 resulting in eight treatments of controlled fermentation. Triplicate samples were withdrawn for analyses at 0, 24, 40 and 65 hours of fermentation for enzymatic and physico-chemical analyses.

Table 1: Research design for multiple starter culture fermentation of *soybean-daddawa*

Sample	**Micro-organism(s) used**
A	*Bacillus subtilis* LB3 + *Staphylococcus xylosus* SAU3 (1% salted)
B	*Bacillus subtilis* LB3 + *Staphylococcus xylosus* SAU3 (salt-free)
C	*Bacillus subtilis LB3+ Leuconostoc mesenteroides* ssp *cremoris* (1% salted)
D	*Bacillus subtilis LB3 + Leuconostoc mesenteroides* ssp *cremoris* (salt-free)
E	*Staphylococcus xylosus SAU3 and Leuconostoc mesenteroides* ssp *cremoris* (1% salted)
F	*Staphylococcus xylosus SAU3 and Leuconostoc mesenteroides* ssp *cremoris* (salt-free)
G	*Bacillus subtilis LB3 + Staphylococcus xylosus SAU3 + Leuconostoc mesenteroides* ssp *cremoris* (1% salted)
H	H *Bacillus subtilis LB3 + Staphylococcus xylosus SAU3 + Leuconostoc mesenteroides* ssp *cremoris* (salt-free)

2.3 Enzymatic and Physico-Chemical Analyses of the Fermenting Soybean Seeds

Enzyme extract of the fermenting soybean seeds was prepared by grinding 5 g of sample in 50 ml of 0.1 M Phosphate buffer, pH 6.5 as the extracting buffer as described earlier (Kolapo et al., 2019b). The extract thus obtained was used for both protease and alpha amylase activity as described by Dakwa et al., 2005 and Kolapo et al., 2019b.

The method described by Odibo et al. (1990) was used to obtain the extract used for free amino acid (FAA) and total soluble sugar (TTS) content assays of the fermenting *soybean-daddawa*. The total free amino acid content was determined by the ninhydrin colorimetric method of Rosen (1957) as described by Dakwa et al. (2005) and the free amino acids were determined by correlating absorbance at 420 nm with a standard of glycine. The total soluble sugar was determined by the anthrone reagent method of Morris (1948) as described by Omafuvbe et al. (2002). The total soluble sugar was determined by correlating absorbance at 625 nm with a standard of glucose.

The method described by Sanni (1988) and Ikenebomeh et al. (1986) were used for the analyses of the pH and titratable acidity of the fermenting *Soybean-daddawa.* Detail procedures have been described earlier (Kolapo et al., 2019b).

2.4 Sensory Evaluation of the Fermented Soybean Seeds

All the eight batches of *Soybean daddawa* samples produced through starter culture fermentation procedures were subjected to organoleptic evaluation. Attributes evaluated include - colour, texture, flavour, stickness, and over all acceptability. The samples were assessed by a panel of 50 regular consumers of *soybean-daddawa* using a 9-point hedonic scale- with score range of 1 (dislike extremely) to 9 (like extremely).

2.5 Statistical Analysis

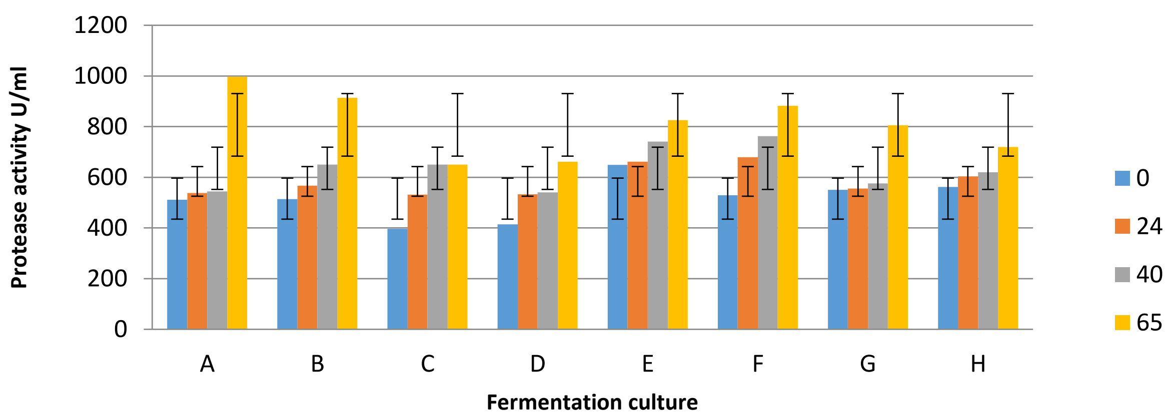
Data obtained were expressed as means ± standard deviation. Analysis of variance was carried out on the data obtained to determine the significance of differences. A two-tailed P value of less than or equal to 0.05 was considered to be statistically significant. Values that were significantly different were separated using the Duncan Multiple Range test using SPSS for Windows Version 17.0 statistical package.

3. Results and discussion

In all the eight experimental setups, protease activity increased significantly ($p \leq 0.05$) between the 0^{th} and 65^{th} hour of the controlled fermentation (Figure 1). However, the pattern of changes depended on the starter culture composition and the inclusion of salt or otherwise. In this connection, at the end of 65 h fermentation, salted soybean seeds fermented with *Bacillus subtilis* LB3 and *Staphylococcus xylosus* SAU3 (Sample A) had the highest protease activity (997 U/ml) while those fermented with *Bacillus subtilis* LB3 and *Leuconostoc mesenteroides* ssp *cremoris* LAB5 (Sample C) had the least value (650 U/ml).

Due to the exceptional proteolytic activity of *Bacillus* strains, the traditional fermentation of *soybean-daddawa* has been reported to be accomplished by mainly by *Bacillus* species, notably *B. subtilis, B. pumilus*, and *B. licheniformis* (Ogbadu and Okagbue, 1988; Omafuvbe et al., 2000; Dakwa et al., 2005). It is therefore not surprising that among the multiple starter cultures used in the present study, those that contained *Bacillus subtilis* LB3 (Samples A and B) demonstrated highest protease activity. The result of this study also indicates that salt inclusion enhanced the activity of protease as the protease activity in sample A was higher than that of sample B. This result is in agreement with the report of Omafuvbe (2006) on *B. subtilis* SDA3 fermentation of 1% salted *soybean-daaddawa*. In a similar trend, Joo and Chang (2005) reported that 1% and 0.4 % NaCl gave maximum protease activity and enhanced protease production respectively in *Bacillus clausii.* Also, the hydrolysis of protein in sufu (fermented soybean curd) have been reported to be influence to a large extent by NaCl, thus resulting in increased level of free amino acids (Han et al., 2003).

Fig 1. Changes in protease activities in salted (1%) and salt-free *soybean-daddawa* fermented with multiple starter cultures for 65 hours



KEY for Fermentation culture
A= *Bacillus subtilis* LB3 + *Staphylococcus xylosus* SAU3 (salted)
B= *Bacillus subtilis* LB3 + *Staphylococcus xylosus* SAU3 (salt-free)
C= *Bacillus subtilis LB3 + Leuconostoc mesenteroides ssp cremoris* (salted)
D= *Bacillus subtilis LB3 + Leuconostoc mesenteroides ssp cremoris* (salt-free)
E= *Staphylococcus xylosus SAU3 and Leuconostoc mesenteroides ssp cremoris* (salted)
F= *Staphylococcus xylosus SAU3 and Leuconostoc mesenteroides ssp cremoris* (salt-free)
G= *Bacillus subtilis LB3 + Staphylococcus xylosus SAU3 + Leuconostoc mesenteroides ssp cremoris* (salted)
H= *Bacillus subtilis LB3 + Staphylococcus xylosus SAU3 + Leuconostoc mesenteroides ssp cremoris* (salt-free)

The α-amylase activity in the fermenting salted and salt-free seeds progressively increased from the onset of the fermentation process till the end of the 65th hour. However, it is worthy to note that at the end of the fermentation, the values for the salt-free fermented product was significantly ($p \leq 0.05$) higher than those obtained from the salted seeds, except for the product obtained using *Staphylococcus xylosus* SAU3 and *Leuconostoc mesenteroides* ssp *cremoris* LAB5 (Sample E) (Figure 2).

The progressive increase of α-amylase activity in the fermenting soybean seeds as fermentation progressed is an indication of progressive hydrolysis of the starch components of the soybean seeds in order to produce monosaccharaides for energy metabolism of the microbes involved in the fermentation (Mohammed, 2021). This trend is in agreement with previous reports on *Soybean-daddawa* fermentation (Omafuvbe et al., 2000, 2002; Dakwa et al., 2005; Kolapo et al., 2019b).

Enache et al. (2009), while investigating the effect of NaCl on the amylase activity of some archaea isolated from Techirghiol Lake, Romania, reported that at low salt concentration, amylase activity was reduced or in most cases was abolished in media of pH higher than 7.0.

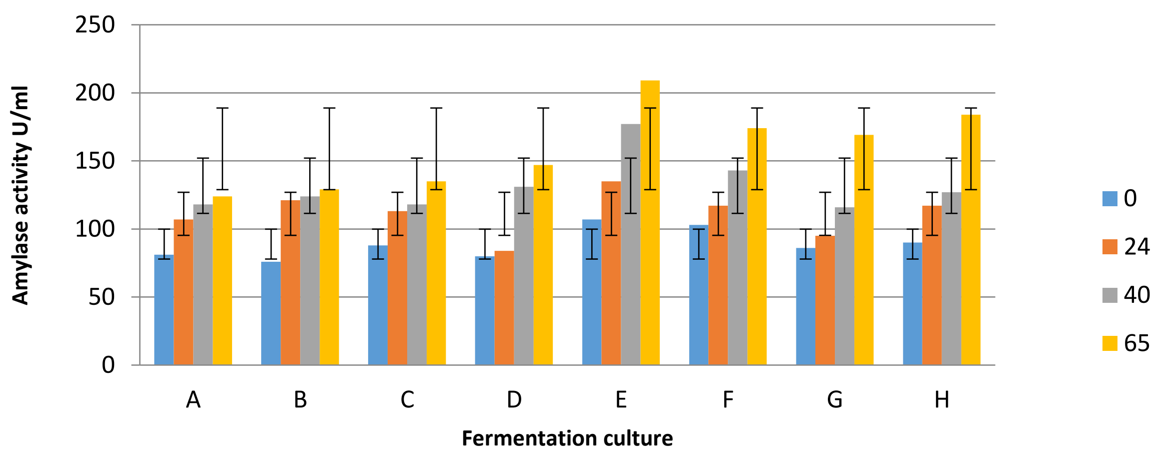


Fig 2. Changes in amylase activities in salted (1%) and salt-free *soybean daddawa* fermented with multiple starter cultures for 65 hours

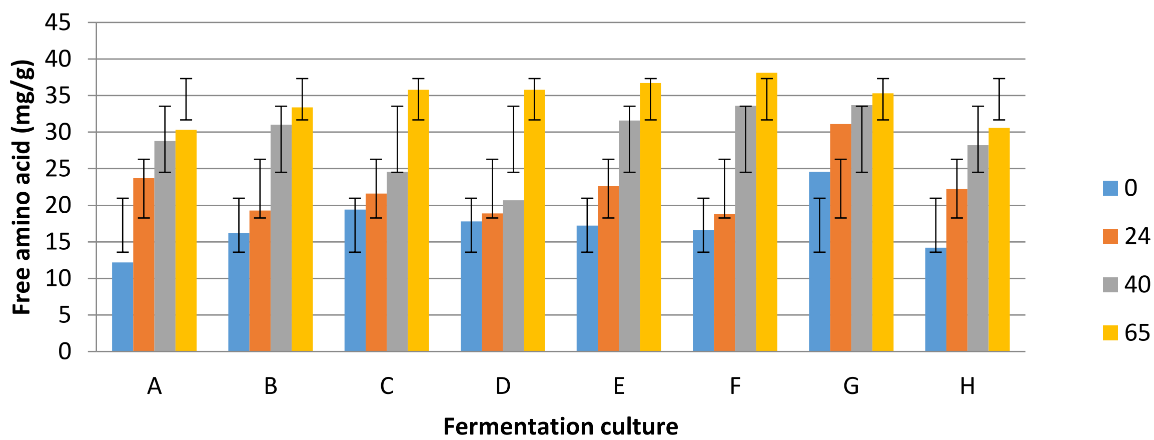
KEY for Fermentation culture
A= *Bacillus subtilis* LB3 + *Staphylococcus xylosus* SAU3 (salted)
B= *Bacillus subtilis* LB3 + *Staphylococcus xylosus* SAU3 (salt-free)
C= *Bacillus subtilis LB3 + Leuconostoc mesenteroides ssp cremoris* (salted)
D= *Bacillus subtilis LB3 + Leuconostoc mesenteroides ssp cremoris* (salt-free)
E= *Staphylococcus xylosus SAU3 and Leuconostoc mesenteroides ssp cremoris* (salted)
F= *Staphylococcus xylosus SAU3 and Leuconostoc mesenteroides ssp cremoris* (salt-free)
G= *Bacillus subtilis LB3 + Staphylococcus xylosus SAU3 + Leuconostoc mesenteroides ssp cremoris* (salted)
H= *Bacillus subtilis LB3 + Staphylococcus xylosus SAU3 + Leuconostoc mesenteroides ssp cremoris* (salt-free)

Though, that report indicated that the amylase activity of these archaea requires neutral or slightly acidic pH values, yet the influence of the source of the microbial enzyme on its activity was brought to bear as the presence of amylase activity at pH >8.0 in other archaea studied was reported in the same study.

Soybean-daddawa fermentation is an alkaline fermentation in which pH >8.0 are attained in the process of fermentation. This alkaline pH coupled with the presence of NaCl could have synergistically resulted in the observed reduction of amylase activity in the multiple starter culture fermentation of salted *soybean-daddawa* as witnessed in the present study. However, this observed reduction of amylase activity may not be a serious cause of concern in alkaline fermented foods such as *soybean-daddawa*. This is plausible as desired flavor characteristics of the end product depends on the metabolism of the amino acids (generated through proteolysis) by the starter cultures to generate wide arrays of volatiles and flavor compounds.

There was a significant ($p \leq 0.05$) increase in the FAA content of the fermenting seeds in all the experimental set-ups. However, the specificity of starter culture composition and presence/absence of salt determined the pattern of the observed increase (Figure 3). At the end of 65h of fermentation, salted seeds fermented with the 'cocktail' culture of *Bacillus subtilis* LB3, *Staphylococcus xylosus* SAU3 and *Leuconostoc mesenteroides* ssp *cremoris* LAB5 (Sample G) only had a value that was significantly ($p \leq 0.05$) different from the salt-free fermented seeds (Sample H) .

Fig 3. Changes in free amino acids contents of salted (1%) and salt-free *soybean daddawa* fermented with multiple starter culture for 65 hours



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C= *Bacillus subtilis LB3 + Leuconostoc mesenteroides ssp cremoris* (salted)
D= *Bacillus subtilis LB3 + Leuconostoc mesenteroides ssp cremoris* (salt-free)
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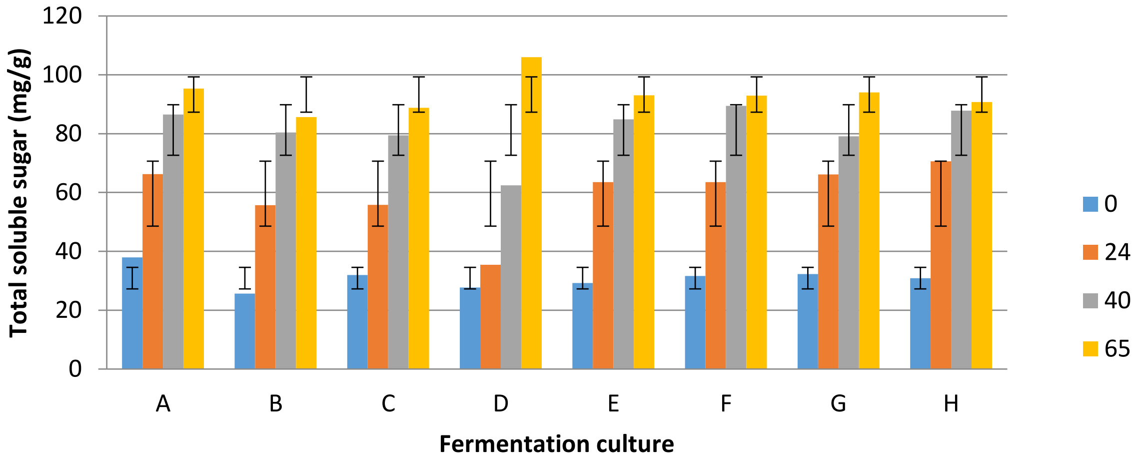
G= *Bacillus subtilis LB3 + Staphylococcus xylosus SAU3 + Leuconostoc mesenteroides ssp cremoris* (salted)
H= *Bacillus subtilis LB3 + Staphylococcus xylosus SAU3 + Leuconostoc mesenteroides ssp cremoris* (salt-free)

The pattern of change in the FAA contents of the fermenting *soybean daddawa* in the present study is in agreement with previous reports of Aderibigbe and Odunfa (1990) and Omafuvbe et al. (2002) whereby increased protease activity resulted in increased FAA contents. As reported by previous authors, the free amino acids thus generated could be utilized by the fermenting bacteria as sources of carbon and energy to produce ammonia (Allagheny et al., 1996) or undergo Strecker degradation of amino acids resulting in the formation of α-amino ketones which in turn can condense with α-dicarbonyl compounds to form pyrazzine (MacLeod and Ames, 1988), which is an important flavour compounds.

In all the fermentation setups, the TTS increased significantly ($p \leq 0.05$) between the 0^{th} and 65^{th} hour of the fermentation process. The observed changes were influenced by nature of the mix of the starter culture and the presence of salt (Figure 4). In most cases, the difference between the values for salted and salt-free fermenting seeds was not significant ($p > 0.05$).

Fig 4. Changes in total soluble sugars contents of salted (1%) and salt-free *soybean daddawa* fermented with multiple starter cultures for 65 hours



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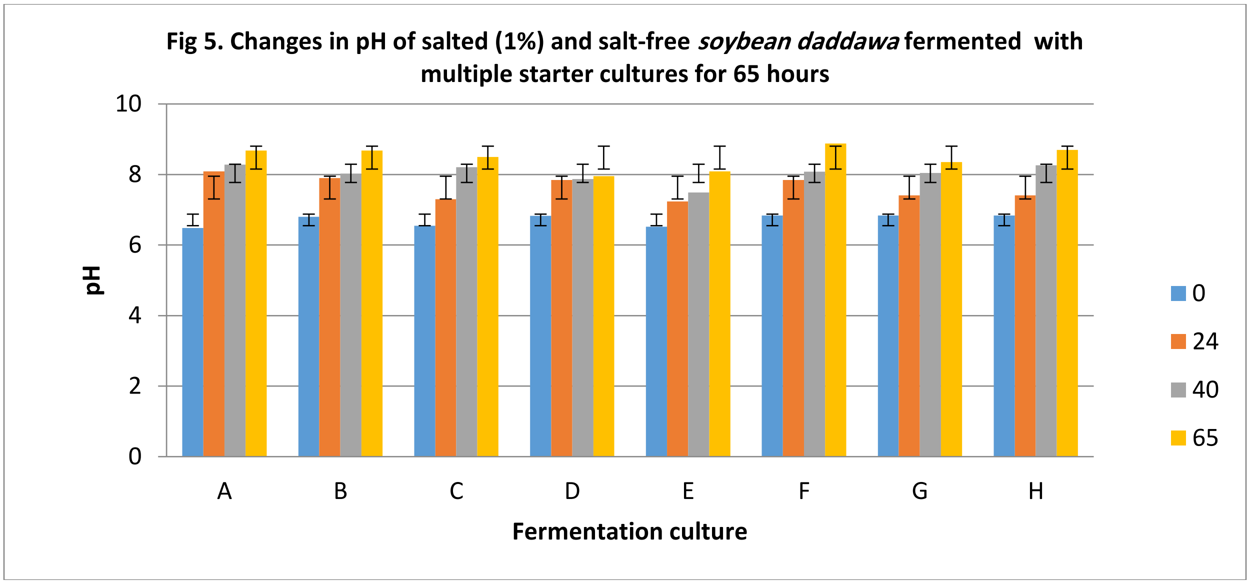
G= *Bacillus subtilis LB3 + Staphylococcus xylosus SAU3 + Leuconostoc mesenteroides ssp cremoris* (salted)
H= *Bacillus subtilis LB3 + Staphylococcus xylosus SAU3 + Leuconostoc mesenteroides ssp cremoris* (salt-free)

This pattern of change in the total soluble sugar (TSS) contents of the fermenting *soybean daddawa* as observed in the present study, whereby the progressive increase in the amylase activity with fermentation time resulted in increased TSS contents, is in agreement with previous reports on salt-free *soybean-daddawa* (Omafuvbe et al., 2000, 2002; Dakwa et al., 2005; Kolapo et al., 2019b) and salted *soybean-daddawa* (Omafuvbe (1994, 2006). The role of these generated monosaccharides in the development of flavour compounds has been documented. Rizzi (1987) and Whitfield (1992) submitted that a number of amino acids readily react with monosaccharides to form alkylpyrazines. Leahy and Reineccius (1989) also suggested that the formation of pyrazines is favoured by free ammonia as Owens et al. (1997) submitted that the combination of alkaline pH (~8.0), high ammonia concentration and free amino acids in the fermentations allowed the formation of pyrazines at the 35^{0}C incubation temperature.

The pH of all the fermenting salted and salt-free *soybean-daddawa* significantly ($p \le 0.05$) increased with fermentation time. Alkaline pH developed much more rapidly within 24 h of fermentation in samples fermented with *Bacillus subtilis* LB3 and *Staphylococcus xylosus* SAU3 (Samples A and B). However, at the end of 65 h fermentation, no significant difference ($p > 0.05$) existed in the pH of all the fermented *soybean-daddawa* irrespective of whether it was salted or not. (Figure 5).



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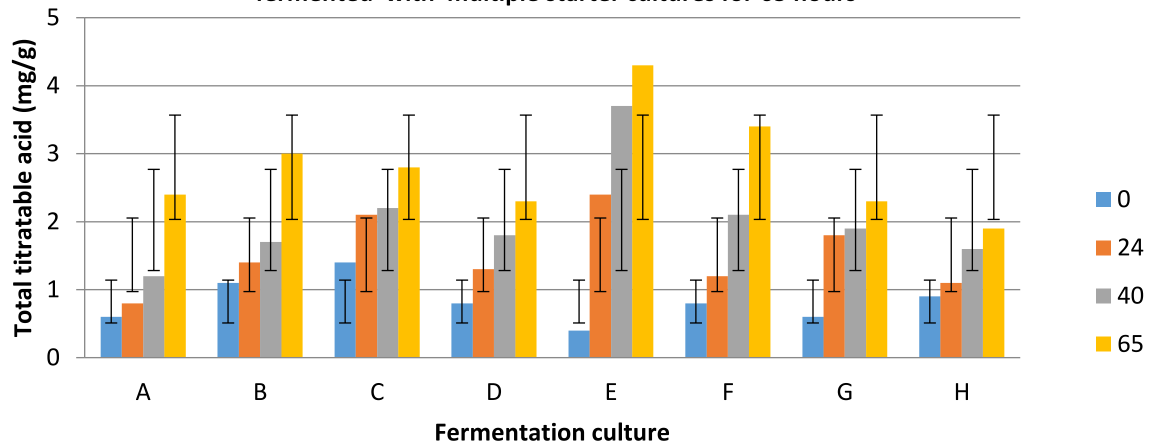
H= *Bacillus subtilis LB3 + Staphylococcus xylosus SAU3 + Leuconostoc mesenteroides ssp cremoris* (salt-free)

The rise in pH with fermentation time as observed in the present study is congruous with previous reports on legume fermentation (Ohta, 1986; Steinkraus, 1991; Sarkar et al., 1993; Omafuvbe et al,. 2002; Kolapo et al., 2019b). The rise in pH was a result of proteolytic activities and the release of ammonia following the utilization of amino acids by the fermenting bacterial culture. The release of ammonia or other basic end products resulted to the development of alkaline pH. The rapid development of alkaline pH within 24 h of fermentation in Samples A and B was akin to previous reports on *B. subtilis* fermentation of *soybean-daddawa* (Omafuvbe 2006; Kolapo et al., 2019b). In those studies, it was suggested that the rapid development of the alkaline pH in *B. subtilis* fermented *soybean-daddawa* within 24 h of fermentation was possibly due to higher rate of metabolism of amino acids liberated by proteolysis to produce ammonia and other basic end products.

Throughout the whole period of fermentation, TTA constantly increased significantly ($p \leq 0.05$) in both fermented salted and salt-free seeds. The fermentation mediated by the mixed culture of *Staphylococcus xylosus* SAU3 and *Leuconostoc mesenteroides* ssp *cremoris* LAB5 (Sample E) resulted in the highest value of 4.3 mg/g lactic acid in the salted seeds (Figure 6). The salt-free seeds fermented with the trios of *Bacillus subtilis* LB3, *Staphylococcus xylosus* SAU3 and *Leuconostoc mesenteroides* ssp *cremoris* LAB5 (Sample H) had the least value of 1.9 mg/g lactic acid.

The development of titratable acidity during alkaline/legume fermentation, as fermentation progressed, is not particularly strange as studies have reported simultaneous increase in pH and titratable acidity with increasing fermentation time (Ikenebomeh, 1989; Omafuvbe *et al*., 2000; Kolapo et al., 2019b). In these studies it was suggested that liberated ammonia or other basic end products of protein decomposition were the cause of pH increase while the metabolism of sugar (obtained through amylolytic hydrolysis of starch) resulted into the formation of organic acids which gave rise to increased titratable acidity.

Fig 6. Changes in titratable acidity of salted (1%)and salt-free *soybean daddawa* fermented with multiple starter cultures for 65 hours



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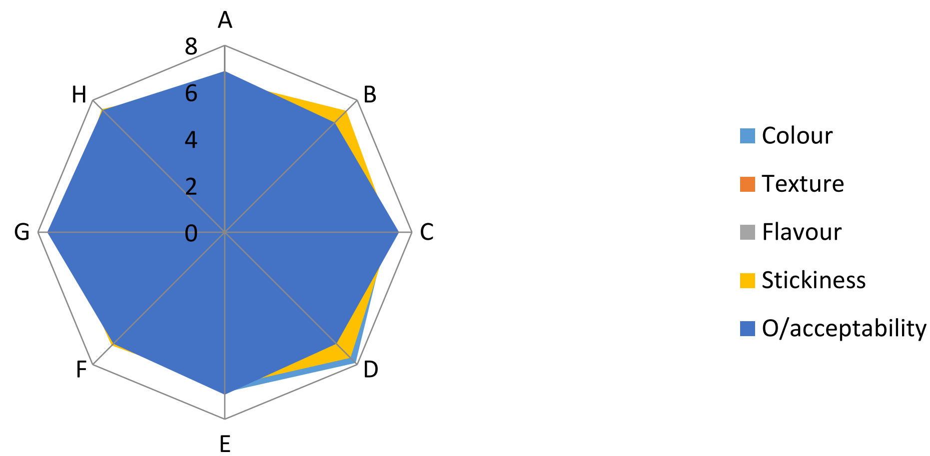
H= *Bacillus subtilis LB3 + Staphylococcus xylosus SAU3 + Leuconostoc mesenteroides ssp cremoris* (salt-free)

The result of sensory evaluation of multiple starter culture fermented salted and salt-free *soybean-daddawa* is shown in figure 7. There was no significant difference in the ratings for both flavor and texture for samples A – H. Similarly, the colour of all samples was rated equally except sample D; likewise the stickiness rating for all the samples was uniform except samples B and D. All the fermented seeds, whether salted or otherwise, were organoleptically acceptable to the *soybean-daddawa* consumers. However, the scores for overall acceptability of the fermented soybean seeds indicated that the panelist demonstrated highest preference for salted *soybean-daddawa* fermented with the'cocktail' culture of *Bacillus subtilis* LB3, *Staphylococcus xylosus* SAU3 and *Leuconostoc mesenteroides* ssp *cremoris* LAB5 (Sample G) and this was closely followed by its salt-free equivalent (Sample H) (Figure 7).

Fig 7. Sensory evaluation of salted (1%) and salt-free *soybean daddawa* fermented with multiple starter cultures



KEY for Fermentation culture
A= *Bacillus subtilis* LB3 + *Staphylococcus xylosus* SAU3 (salted)
B= *Bacillus subtilis* LB3 + *Staphylococcus xylosus* SAU3 (salt-free)
C= *Bacillus subtilis LB3 + Leuconostoc mesenteroides ssp cremoris* (salted)
D= *Bacillus subtilis LB3 + Leuconostoc mesenteroides ssp cremoris* (salt-free)
E= *Staphylococcus xylosus SAU3 and Leuconostoc mesenteroides ssp cremoris* (salted)
F= *Staphylococcus xylosus SAU3 and Leuconostoc mesenteroides ssp cremoris* (salt-free)
G= *Bacillus subtilis LB3 + Staphylococcus xylosus SAU3 + Leuconostoc mesenteroides ssp cremoris* (salted)
H= *Bacillus subtilis LB3 + Staphylococcus xylosus SAU3 + Leuconostoc mesenteroides ssp cremoris* (salt-free)

Achi (2005) had earlier opined that the use of mixture of microorganisms with complimentary physiological and metabolic properties seems to be the best approach for obtaining a product with the nutritional and sensory properties desired in indigenous African fermented foods produced by starter culture fermentation. In this connection, it had been demonstrated that there is a need for a paradigm shift from the restrictive use of *Bacillus* strains to the use of 'cocktail' cultures in order to replicate the traditional natural aroma and acceptability during the controlled fermentation of *soybean-daddawa*. (Kolapo et al*.,* 2019 a, b).

4. Conclusion

The results of this study suggest that the addition of 1% NaCl improved the organoleptic quality of *soybean-daddawa* produced with the 'cocktail' culture of *Bacillus subtilis* LB3, *Staphylococcus xylosus* SAU3 and *Leuconostoc mesenteroides* ssp *cremoris* LAB5 (Sample G). The salt, at the investigated concentration of 1%, enhanced protease activity of the starter cultures and subsequent production of amino acids which are considered to be important flavor enhancing compounds in many fermented foods. However, there was an observed reduction of amylase activity and total soluble sugars in the multiple starter culture fermentation of salted *soybean-daddawa.* Addition of salt exerted no significant effect on the pH of the fermented *soybean-daddawa.* The present report represents an important milestone in the quest for industrialization of *soybean-daddawa* production more importantly that the 1% salt concentration is within the adopted salt reduction strategies in many regions of the world.

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