The microbiology and proximate assay of a novel weaning food—‘DUPAP’

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ABSTRACT

A novel weaning food, ‘Dupap’ containing varying quantities of pap and African yam bean(‘Odudu’) (Sphenostylis stenocarpa -Harms) was developed. Microbiological analysis of the food revealed the presence of species of Aspergillus, Rhizopus, Candida, Penicillium, Staphylococcus, Bacillus, Streptococcus, Neisseria, Proteus, and Lactobacillus species. Total heterotrophic bacterial and fungal counts revealed 3.0x10^7, 3.2x10^5 cfu/ml and 1.0x10^7, 0.8x10^3 cfu/g respectively for wet and dried pap. The proximate analyses revealed that the protein and fat contents of maize decreased from 10.33% to 7.0% and 4.24% to 0.46% respectively after processing for pap; carbohydrate levels increased from 80.16% to 90.14%. Carbohydrate and protein contents of raw African yam bean decreased progressively after steeping and pulverization into flour. The proximate quality of different blends of ‘Dupap’ revealed progressive increase as the percentage of African yam bean increased, except the carbohydrate level. There was no significant difference in the sensory attributes of the ‘Dupap’ blends (P≤ 0.05).This study concludes that ‘Dupap’ is a weaning food, meeting acceptable levels for protein in weaning foods.

Key words: microbiological analysis, weaning food, ‘Dupap’, proximate quality, sensory attributes.

INTRODUCTION

Fermented foods are consumed by a large number of people in different parts of the world (Steinkraus, 1983). Fermented foods play a major role in developing countries by providing essential nutrients and variety in diet. They offer improved taste, flavor, digestibility and reduction of antinutritional qualities, among others. Fermented foods are usually prepared from plant or animal materials by processes in which microorganisms play important role of modifying the substrates physically, nutritionally and sensorily (Aidoo, 1986; Otitoju, 2009). Considering the high cost of animal protein from such sources as meat, milk, fish and eggs, fermented foods are of immense value as protein supplements (Banigo et al., 1986; Nnanyelugo and Onofok, 1998), especially in developing countries with very little per capita income. In Nigeria, fermented foods constitute a major part of the diet (Odunfa, 1981). These include those from cereals (e.g maize, millet, sorghum etc), legumes (e.g soybean, cowpea), fruits (e.g oil bean seed), tubers (e.g garri, ‘fufu’, tapioca etc) and tree sap (e.g palm wine). Less popularly used legumes include the African yam beans (Sphenostylis stenocarpa -Harms)( Njoku et al., 1990).

Ogi or Akamu or pap is an important weaning food in Nigeria (Akinrele, 1970; FAO, 2006). It is prepared from steeped, ground, sieved and fermented dry maize seed. It could also be made from sorghum or millet. It is not only used as a weaning food but may also be consumed by adults as a breakfast dish in combination with akara (Cowpea buns) or fried ripe plantain. This fermented maize meal has been found to have such low nutritional value that it did not support the growth of rats (Akinrele, 1966; Abdulrahaman and Kolawole, 2006). This is because corn (or other cereals) used in the preparation of pap is deficient in essential amino acids such as lysine and tryptophan.
al., 1976, Teniola and Odunfa, 2001). Therefore, infants fed exclusively on pap during weaning have high tendency of developing protein malnutrition.

The socio-economic status of the Nigeria people, population explosion and poor education on proper feeding habits affect the nutritional status of babies of weaning age 6-12 months (Fasoyiro et al., 2002; Johnson, 2003). Effects of malnutrition on babies are on the increase in poor countries of the world. Insufficient intake of proteins, calories, vitamins and minerals predispose babies to frequent infections, and also cause malnutrition. Such illnesses as diarrhea, measles, malaria, and respiratory disease tax the body heavily and cause loss of nutrients. They reduce appetite and food intake, thus contributing to malnutrition.

Recent studies have been carried out in an effort to improve the nutritional quality of carbohydrate-based foods (Achinewhu et al., 1992; Akpapunam, 1985). Often, protein from other sources are used to enrich cereal-based diets (Adeyemi, 1991; Isu et al., 1994). For both nutritional and economic reasons, there is a dire need to fortify indigenous foods with lesser used legumes with a view to solving the malnutrition-related problems that have contributed to increasing mortality and morbidity rates in developing countries of Africa.

Microorganisms involved in the fermentation of most cereal and legume-based foods have been investigated (Akinrele, 1970; Omemu and Omeike, 2006; Akharaiyi and Omoya, 2008). These include lactic acid bacteria, Bacillus spp, and Staphylococcus spp.

Having considered the increasing cases of malnutrition of children of weaning age (6-12 months) in developing countries attributed to the high cost and non-affordability of conventional weaning foods (i.e. those rich in protein and minerals), this study is aimed at developing a novel weaning food ‘Dupap’. ‘Dupap’ combines the attributes of being affordable, rich in proteins and minerals, 100% local content and having organoleptic characteristics as the major weaning food (‘Ogi’) in Nigeria and some other African countries.

MATERIALS AND METHODS

African yam bean seeds (Sphenostylis stenocarpa - Harms) and maize (Zea mays) were obtained from the Afor-Oru market in Ahiazu mbaise local government area of Imo State, Nigeria.

Preparation of Pap (‘Ogi’):

Pap was made as described by Banigo et al., (1974). The maize seeds were sorted and washed, steeped to ferment for 72 hours, and the water drained. The fermented grains were wet milled and wet sieved. The slurry was allowed to stand, ferment and settle overnight. The supernatant was drained off, while the slurry (‘ogi’) was boiled to make ‘ogi’ porridge.

Preparation of Odudu (African yam bean) flour:

The African yam bean seed was processed as described by Njoku et al., (1991). The seeds were cleaned, washed and steeped for 12 hours. The water was drained off, while the seeds were dehulled, cooked at 100°C for 45 minutes and dried at 57°C for 18 hours in an oven. It was subsequently ground into flour using crown dry miller.

Microbiological analyses:

The total heterotrophic count was determined using Oxoid nutrient agar and Sabouraud dextrose agar (SDA) for bacteria and fungi respectively. A 0.1g/l of chloramphenicol was added to SDA to inhibit the growth of bacteria. An aliquot of 0.1 ml of 10^6 dilution of each sample was inoculated aseptically into labelled duplicate agar plates and incubated at 37°C for 24-48 hours and at room temperature for 24-96 hours for bacteria and fungi respectively. Isolated microorganisms were characterized and identified using cultural and morphological characteristics as well as biochemical tests (Cheesbrough, 2003).

Proximate analyses of nutrients:

The analyses were carried out according to the method described by James (1995). The percentage crude protein, crude fibre, extractable lipid, ash and carbohydrate contents in Sphenostylis stenocarpa- Harms raw seed, after steeping and seed after dehulling were determined. This was also carried out for the raw maize, after steeping and the pap (Ogi). The proximate quality of the different blends of pap and ‘Odudu’ were also determined.

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Sensory evaluation of the different blends of ‘Dupap’.
The different blends (proportion of paps and ‘Odudu’ flour) were evaluated organoleptically for taste and acceptability. The panel consisted of 10 members aged between 26 and 40 years. The panelists were asked to taste each blend, after which they recorded their score on the evaluation form provided. Crackers biscuits and water were provided for rinsing their mouth after tasting each sample. A nine-point Hedonic scale was used to assess the taste and acceptability (Njoku et al., 1991).

RESULTS

The microbiological analyses of replicate samples of ‘Odudu’ steep water, mashed maize, pap (‘Ogi’) and ‘Odudu’ flour revealed the presence of Staphylococcus, Neisseria, Proteus, and Lactobacillus species. The highest total heterotrophic count for bacteria was 3.0x10^8 cfu/ml obtained for ‘Odudu’ steep water; followed by maize steep water (2.7x10^6 cfu/ml), mashed maize (2.5x10^6 cfu/ml), dehulled ‘Odudu’ seed (2.0x10^6 cfu/ml), wet ‘Ogi’/pap (3.0x10^7 cfu/ml), ‘Odudu’ flour (2.0x10^7 cfu/g) and dried ‘Ogi’/pap (1.0x10^7 cfu/g). The highest fungal load of 3.2x10^5 cfu/ml was obtained from wet pap/‘Ogi’. It was followed by mashed maize, maize steep water, odudu steep water, dehulled odudu and dried pap/‘Ogi’ in that order (Fig 3.0). No fungal isolate was recovered from ‘Odudu’ flour. Amongst the fungal genera, Aspergillus niger occurred in 83% of the samples. Aspergillus niger occurred in 67% of the samples whereas Rhizopus and Penicillium occurred in 50% and 33% of the samples respectively (Table 1.0). For the bacterial isolates Streptococcus and Bacillus occurred in 100% of the samples, Neisseria and Lactobacillus occurred in 83% of the samples while Proteus and Streptococcus occurred in 67% of the samples. ‘Odudu’ steep water and maize steep water contained all (100%) of the genera of bacteria isolated in this study. Mashed maize contained 75% of the fungal isolates, dehulled ‘Odudu’ seed contained 50%, pap/‘Ogi’ 25%, whereas ‘Odudu’ flour harboured no fungal isolates. Amongst the bacterial flora, dehulled ‘odudu’ seed had 66.66% of the isolates whereas ‘odudu’ flour had 33.33% of the isolates. These are shown on Table 2.

Table 1.0 Microbial Flora (Fungi) in Test Samples

<table>
<thead>
<tr>
<th>S/No</th>
<th>Sample</th>
<th>Aspergillus niger</th>
<th>Rhizopus</th>
<th>Candida</th>
<th>Penicillium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mashed maize</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>2</td>
<td>Maize steep water</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>3</td>
<td>‘Ogi’</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>4</td>
<td>Dehulled odudu seed</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>5</td>
<td>‘Odudu’ steep water</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>6</td>
<td>‘Odudu’ flour</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>

| 4(67%) | 3(50%) | 5(83%) | 2(33%) |

Table 2 Microbial Flora (Bacteria) in test samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Staph</th>
<th>Bacillus</th>
<th>Strept</th>
<th>Neiss</th>
<th>Prot</th>
<th>Lact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mashed maize</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Maize steep water</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>‘Oi’</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Dehulled odudu seed</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>‘Odudu’ steep water</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>‘Odudu’ flour</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>

| 6(100%) | 6(100%) | 4(67%) | 5(83%) | 4(67%) | 5(83%) |

Proximate analysis

The result of proximate analysis of the raw test samples and the different blends of ‘Dupap’ are shown in Figures 2 and 3 respectively. The proximate analysis at different stages as shown in revealed that there was a sharp decrease in the fibre content of raw ‘odudu’ seed from 3.66% to 3.42% after steeping. This increased to 3.48% after dehulling and later increased to 3.62% when pulverized into flour. On the other hand, raw ‘Odudu’ had an ash content of 300.
4.96% which decreased to 3.88% after steeping. After dehulling, the ash content increased to 4.68% while a slight decrease to 4.66% was observed after pulverization into ‘odudu’ flour (Fig 2.0). The effect of processing on fat levels revealed that the raw seed contained 1.98% fat. This decreased progressively to 1.86% and 1.92% after steeping and dehulling respectively, and increased to 2.02 % after pulverization into flour. The carbohydrate levels decreased progressively from 61.57% to 60.30% at the end of pulverization. The protein content also decreased progressively from 29.40% from the raw stage, to 27.83% after pulverization into flour(Fig 2.0). The result also revealed that the protein content of the raw maize seed (10.33%) decreased to 7.0% at the end of pap/’ogi’ production. The fat and carbohydrate levels also showed a linear trend from 4.24% to 0.46% and 80.16% to 90.14% respectively from the raw maize to the final pap/’ogi’ product. However, the fibre and ash contents showed a sharp decrease from the raw maize of 1.46% and 3.82% to 1.08% and 1.32% respectively at the end of pap production (Fig 2.0). On the other hand, the proximate quality of the different blends of ‘Dupap’ revealed a progressive increase (Figure 3.0) as the percentage of ‘Odudu’ increased, for protein, ash, fat, and fibre. The carbohydrate levels showed a decreasing trend as the percentage of ‘Odudu’ increased in the blends.

**Statistical analysis**

The analysis of variance for the sensory attributes in relation to the different blends of ‘Dupap’, all revealed non-significant treatment effects at P≤0.5 (i.e., colour, general acceptance, texture, taste and aroma or flavor). Analysis of the different blends in relation to the sensory attributes revealed no significant effects at 5% level of significance, for 0%, 5%, 10%, 40%, 60% and 80% of ‘Dupap’. However, there was significant treatment effect in the 20% ‘Dupap’ blend. Further analysis for 20%, using the least significant difference at P≤0.5 revealed that colour did not differ significantly from texture. Also flavor and taste are not significantly different. However, general acceptance and taste together differed significantly from colour and texture, which means that general acceptance does not depend on taste alone, or colour, or texture.

**DISCUSSION AND CONCLUSION**

A total of ten isolates (six bacterial genera, and four fungal genera) were recorded in this study. The isolation of *Streptococcus* species and *Lactobacillus* species from pap corroborates the report of Steinkraus (1983), and Ozoh and Kuyanbana (1995). Total heterotrophic bacterial counts revealed high aerobic plate counts ranging from 1.0x10^7 cfu/g in dried pap, to 2.0x10^7 cfu/g in odudu flour. This is in line with previous findings that indigenous fermented foods sold in Nigeria have high aerobic plate counts (Omuora et al., 1987; Umoh et al., 1989). These high counts may indicate a potential hazard to consumers. These counts are however lower than 1.0x10^5 cfu/ml staphylococcal count normally considered as potentially hazardous (Bergdoll, 1979; Edson et al., 1981). Of the six test samples subjected to microbiological analysis, *Candida* sp, *Staphylococcus* sp, and *Bacillus* sp were isolated from all the samples. The consistent occurrence of *Candida, Staphylococcus* and *Bacillus* can be associated with the role they play in the fermentation processes. Studies on yam bean processing, adopted in this study also revealed the isolation of *Candida* and *Bacillus* with other lactic acid producing bacteria (Ogbulie, 1991). Studies on pap production as reported by Steinkraus et al., (1983) also listed *Candida, Staphylococcus* and *Bacillus* amongst the characteristic bacteria that play obvious roles in pap production. The source of these microorganisms could be human skin, cooking utensils, processing equipment and water (Holt, 1984; Ogbulie, 1991; Njoku et al., 1991; Frazier, 1983). Apart from *Staphylococcus* which has a pathogenic coagulase positive group, most of the other isolates are non-pathogenic and may not be of public health concern. The isolation of other microorganisms such as *Neisseria* sp, *Lactobacillus* sp, *Streptococcus*, *Proteus, Aspergillus niger, Rhizopus*, and *Penicillium* corroborates the report of other studies (Steinkraus et al., 1983; Onyekwere and Akinrele, 1977; Isu et al., 1994). *Lactobacillus plantarum, Streptococcus lactis* and *Saccharomyces cerevisiae* have been reported as predominant microorganisms involved in the fermentation of maize to pap (Iwuoha and Eke, 1996). These isolates may also have been introduced from the environment (Frazier, 1983), and processing equipments (Ogbulie, 1991; Njoku et al., 1991) and or from seedlings used (Obi, 1991). Generally the low occurrence of fungal isolates (molds) could be as a result of acid fermentation that normally takes place during the steeping of maize and African yam bean seedlings.

Proximate analyses were carried out on blends of ‘Dupap’ containing 0% to 100% ‘Odudu’ and pap flours. The most pronounced effect of ‘Odudu’ supplementation on the proximate composition of pap was an improvement in protein content. Fortification of pap with African yam bean resulted in progressive increase in protein content. ‘Odudu’ flour, alone had 30% protein. Similar protein levels have been reported during soybean supplementation of a Kenyan maize meal (Nyotu et al., 1986), and in the studies on diets with ‘Ugba’ (African oil bean) and breadfruit flours (Isu et al., 1994). The protein contents of the blends of pap and ‘Odudu’ flours, from 5% ‘Odudu’.

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flour to 80% ‘Odudu’ flour provide more than 11-14% food protein content recommended for the adequate growth of children (Beaton and Swiss, 1974; Awada et al., 2005). ‘Odudu’ flour had about four times the protein content of pap flour. The total available carbohydrate decreased with increasing ‘Odudu’- supplementation, while the percentage fat increased with increasing ‘Odudu’-supplementation. This also corroborates the report of Nyotu et al. (1986). The percentage ash and fibre contents of the blends also increased progressively with ‘Odudu’-supplementation (Fig.3). Sensory evaluation of the blends revealed that they were highly acceptable in organoleptic properties. The colour, texture, flavor, taste and general acceptability score of the blends were not significantly different (P<0.05). Also analysis of variance results for the sensory attributes show that ‘odudu’/pap blends (0:100), (5:95), (10:90), (40:60), (60:40) and (80:20) respectively were not significantly different (P<0.05), except ‘odudu’/pap blend (20:80) which showed a significant difference (P<0.05).

![Fig.1.0: Total Heterotrophic Count from Test Samples](image1)

*The values on Y-axis are in Cfu/ml x10^8 for bacteria and 10^5 for fungi.*

![Fig.2.0: Proximate Contents of Raw Test Samples](image2)

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In conclusion, a novel weaning food meeting the food protein nutritional content for children has been made. The results of our analyses revealed that ‘Dupap’ blends, up to 40% supplementation (40:60) gave protein level of about 25% which is more than 11-14% protein content recommended for children (Johnson, 2003). Levels of supplementation can vary and will depend on the individual (since they are all sensorily acceptable). The quantity of these foodstuffs available at any given time may also play a role. ‘Odudu’ could therefore improve the nutritional quality of pap, a maize meal, which serves as a predominant weaning food in many parts of Africa including Nigeria. The use of ‘Odudu’ will increase the utilization of ‘Odudu’ (African yam bean), a lesser used legume.

REFERENCES


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