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Abstract: The beany flavour of soya bean has been a major hindrance to the acceptability and consumption of soymilk. Several studies to reduce or eliminate this not very pleasant flavour has been on going. This study was therefore carried out to formulate a drink *Pinasoy* with different blending proportions of pineapple juice and soymilk with the aim of suppressing the “beany” flavour associated with soymilk. The blends consist of these combinations: 80/20, 70/30/60/40, and 50/50 soya milk and pineapple, respectively, with two controls 100% pineapple juice and 100% soya milk. The different blends were formulated, homogenized, blended and packaged for organoleptic evaluation. The microbial load and identification were determined using the streak plate technique of isolation. Bacteria and fungi counts, biochemical tests and microscopic characters of the isolates were carried out using standard techniques. Results showed the taste of 100% pineapple and 80/20% pineapple/soy blend were the most preferred, while the least preferred was the 100% soya milk with a mean value of 1.90. The result of the colour showed the 80/20% blend was the most preferred (4.58) and the least preferred was the 70/30% blend (3.37). In aroma and thickness, the 100% pineapple was the most preferred (3.42) and 3.63, respectively. The least preferred was the 100% soya milk in aroma (2.80) and 60/40% blend in thickness (3.00). On the overall acceptability *Pinasoy* drink prepared from 80/20% pineapple/soy blend was the most accepted (3.63), while the least accepted was the 60/40% blend (2.90). The micro-organisms isolated showed the bacteria count ranged from 5.7×10^4 to 7.45×10^5 cfu/ml, while the fungi count ranged from 1.65×10^4 to 2.64×10^5 cfu/ml. Gram negative bacteria were identified, and they include: *Bacillus spp.*, *E. coli*, *Staphylococcus aureus*, amongst others. The fungi, *Aspergillus spp* and *Penicillium spp* were also identified amongst others.

Keywords: Organoleptic, shelf life, *Pinasoy*, microbiological, pineapple-soya milk blend

Introduction

The prevailing economic trends in Nigeria have made it difficult for the low-income earners to afford foods of animal origin because of their relatively high price and high demand by the high-income earners, and this has led to a great nutrient imbalance which in turn causes malnutrition. Protein, especially of animal origin is required in great proportions, however, its acquisition is not cost-effective for low income earners, and as such plant protein has been used as a substitute. This has resulted to Soya protein flavours being found in dairy proteins (Russell *et al.*, 2006). Most of the world's nutrition-related diseases including birth defects, mental and physical retardation, weakened immune systems, blindness and at times death are a direct result of the consumption of diets lacking in proteins, vitamins and minerals and other micronutrients. As a result of the importance of these micronutrients for good health across all age groups, there have been rising health concerns in recent times of the need to promote the consumption of natural drinks rather than artificial beverages. A number of fruits and legumes such as soybeans have been used as a raw material for natural drink production (Ebahmiegbekho *et al.*, 2016; Singh *et al.*, 2008). Studies have reported soybean to be an excellent dietary source of protein and essential fatty acids, in addition to being naturally low in saturated fatty acids (Messina, 2014; Nagata *et al.*, 2014). It is also relatively cheap in comparison with other food protein sources. Epidemiological studies as well as those using cell cultures, animal models and clinical trials over the last 30–40 years, have suggested that soybean consumption is related to various beneficial health effects (Setchell and Cassidy, 1999; Friedman and Brandon, 2001; Messina, 2001; Xiao, 2008; Nagata *et al.*, 2014; Messina, 2014).

In Asian countries soybean is used as fermented and non-fermented food stuff such as soy sauce, miso, natto, yogurts, kinako, protein crisp, desserts, baby food and soy milk which is further processed into *tofu*, *aburage* and *yuba* (Hammond and Jez, 2011). In the recent update of the ‘dietary guidelines’,

the United States of America government's evidence-based nutritional guidance to promote health and reduce the risk of chronic diseases and the prevalence of obesity through improved nutrition, recommended increasing soy intake as fortified beverages and other soy products (USDA, 2010; Kumar, 2013). While Eastern countries populations consume 20–40 g of soy foods daily, however, the consumption in Western countries is lower than 3 g daily (Kreijkamp-Kaspers *et al.*, 2004; Song *et al.*, 2007; Michelfelder, 2009). Soy beverages are an easy, practical and palatable way to improve soy intake in the Western countries, and their acceptability increases when fruit concentrates or natural fruit juices and other flavours are added (Rodrigues and Moretti, 2008; Felberg *et al.*, 2004). Pineapple (*Ananascomosus.*) being one of the most popular and important commercial fruit crops with several health benefits with its excellent flavour and taste is known as the queen of fruits (Hossain *et al.*, 2015; Silva *et al.*, 2015). Pineapple is reported to contain minerals such as Calcium, Chlorine, Phosphorus, Sodium, potassium, copper, manganese, calcium, magnesium, and very dense in vitamin C, β -carotene, thiamin, B6, folate, as well as soluble and insoluble fibre and bromelain (Faridand Shaheen, 2015; Ferreira *et al.*, 2016). Several methods have been established in order to reduce the “beany” flavour in soymilk. Earlier studies show that less-acidic fruits such as pineapples can work to cover any beany essence, thus resulting in more acceptable products (Hazen, 2007). Ebahmiegbekho *et al.* (2016) had evaluated the use of pineapple juice as a way to reduce this “beany” flavour in soymilk and found the products more acceptable to young adults. The introduction of pineapple juice as an inducer of its tender flavour and sweetener greatly enhanced the level of consumption (Ebahmiegbekho *et al.*, 2016). Therefore the present study was to produce a pineapple-soybean drink blend “*Pinasoy*”, determine the microbiological status of *Pinasoy* and the shelf stability of the final product.

Materials and Methods

The study was carried out at the Food Science and Nutrition Unit, Department of Animal Science Laboratory of the Faculty of Agriculture, University of Benin, Benin City, Edo State, Nigeria.

Processing of pineapple into juice

Fresh and fully ripe Pineapple fruits were purchased from a local market in Benin City and the fruits were washed and rinsed thoroughly with clean, potable water. After the removal of the peel, the fruit was ready for extraction of juice by cutting the pineapple into smaller pieces for ease of blending using a juice blender. The extracted juice was filtered by passing through a sterile muslin cloth into a clean transparent plastic bowl.

Production of soymilk

Soy milk for this study was prepared as described by Yadav, Roshanlal and Parul (2017). Clean soybeans were also purchased from New Benin market and then processed into soymilk. The processing of the soy beans into milk was carried out in stages. The soybean was sorted, cleaned. 1 kg of already sorted soybeans was soaked in water (soybean:water, 1:6) for 8-12 h and the soybean seeds were dehulled manually, washed and blended with water at 30°C to form slurry. The slurry was diluted with about 1.5 litre of clean water to obtain a homogenous slurry and thereafter filtered to have the soymilk. The soymilk was then pasteurized at 70°C for 15 min and was stirred continuously to prevent the product from burning. The milk was then filled hot into already sterilized bottles, cooled and refrigerated.

Pinasoy formulation and organoleptic assessment

Pinasoy was formulated by blending different proportions of pineapple juice and soya milk. Pineapple Juice and soymilk extract were mixed in proportions with the following ratios: 100:0, 80:20, 70:30, 60:40, 50:50, 0:100, to obtain the final product. This was carried out using a food blender operated at full speed for 10 min. The resulting blends were homogenized using improvised equipment; Panasonic blender and pasteurized at 72°C for 15s, hot-filled into sterile bottles, cooled to room temperature (28±2°C) and then stored in a refrigerator at 4°C until analysed. The prepared beverages were evaluated using 5-point hedonic scale, for thickness, colour, taste, aroma, and overall acceptability.

Microbiological analysis

One (1) ml of sample was pipette into 9 ml of sterile water; serial dilution was carried out to 10⁻³. One (1) ml of the sample was pour plated with nutrient agar from the different dilutions. The plates were then inverted and incubated at 37°C for 24 h. The plates with countable colonies were chosen and reported (Jay, 2003). Population of bacteria was obtained by multiplying the number of colonies by the dilution factor. Total viable counts of microorganisms in the Pinasoy were carried out using the Pour Plate technique, where 0.1 ml of the appropriate dilution was plated out on nutrient agar plates. The plates were incubated at 35°C for 48 h and colony forming units per millilitre sample (cfu/ml) were estimated. For fungal counts, the above procedure was repeated using potato dextrose agar and incubation was carried out at 28°C for 3 - 5 days.

Isolation, characterization and identification of pure cultures isolates

Different distinct colonies from the incubated plates were isolated into pure culture. The cultural characteristics such as shape, size, pigmentation, and nature of margins were observed and recorded. This was followed by microscopic examination of cell types.

Identification using MaConkey agar

MaConkey broth was prepared according to the manufacturer's instruction, sterilized by autoclaving at 121°C for 15 min. The media were aseptically poured into plates and

allowed to solidify. The Agar was then plated in duplicates, inverted and incubated at 37°C for 24 h. Lactose positive organisms fermented the medium while non-lactose positive organisms did not ferment the medium (Jay, 2003).

Identification using gram staining method

Gram staining procedure was carried out by fixing the organism to the slide and staining with crystal violet for 20 min after which it was gently rinsed with water, it was then covered with iodine which served as a mordant and then poured off after which it was decolourized with 95% alcohol. It was then rinsed with water to stop the action of the alcohol. The slide was then covered with safranin for 20 secs after which it was gently rinsed off. The slide was blot dry with bibulous paper and observed under the microscope for Gram positive or Gram negative microorganism (Jay, 2003).

Fungi enumeration and identification

Potato dextrose agar was prepared and incorporated with antibiotics (chloramphenicol) to stop the growth of bacteria. Samples from the different dilution were pour plated in duplicates, inverted and incubated at 28°C for 5 days. The plates with countable colonies was chosen and reported.

The population of fungi was calculated by multiplying the number of colonies by the dilution factor. Population was reported to 2 significant figures by Jay (2003).

Colony morphology from the plates was compared with an atlas of fungi and reported. Staining with lacto phenol cotton blue dye to observe spores and shape to further confirm the result from cultural morphology was done.

Statistical Analysis

The data obtained were subjected to statistical analysis using Two Way Classification Analysis of Variance. The values of critical differences at 5% probability level were also calculated using the least significant difference (LSD).

Results and Discussion

Organoleptic (sensory) characteristics of Pinasoy

The mean scores for aroma ranged from 2.79 to 3.42 against 3.40 for the control A (100% pineapple juice). Treatment C (80%/20% pineapple/soymilk), D (70%/30% pineapple/soymilk), E (60%/40% pineapple/soymilk), and F (50%/50% pineapple /soymilk) had a similar aroma to the control A as there was no significant difference ($p>0.05$) recorded. Soybeans confers what is generally termed "beany flavor" which has been shown in earlier studies (Buono *et al.*, 1990; Osundahunsi *et al.*, 2007) to be a major inhibition to the consumption of soy products. The score for aroma of Pinasoy samples reduced gradually as the amount of soymilk added and consequently increased the characteristic "beany" flavour and this has been a major hindrance in the consumption and acceptability of soymilk. The nutritional and health benefits of soybeans cannot however be over stretched (Messina, 1999, 2010; Zhou, 2004). But the increase in the amount of pineapple juice added is lowering the flavour and at the same time, retaining high nutritional quality and acceptability among consumers. The result showed a decrease in mean colour scores from 4.58 at 100% level of pineapple juice to 3.37 at 70% pineapple and 30% soymilk level. This is an indication that soya milk reduced the colour significantly ($p>0.05$). The 100% pineapple juice had the highest mean sensory score in colour (Table 1). This is in contrast to the findings of Akubor (2016), who reported that yoghurt had the highest mean score in the yoghurt-pineapple juice blend. The Pinasoy gradually became more like the control 'B' soymilk in colour. The milky nature of the soymilk became more present than the golden yellow colour of pineapple juice. Earlier reports (Boles and Pegg, 2005; Dias *et al.*, 2012) have shown colour as an important factor in accepting how food is displayed and sold, and thus it is an index used in quality control. Colour is also added to foods to stimulate the appetite

of the consumers (Boles and Pegg, 2005). There was no significant difference ($p > 0.05$) recorded in thickness of the controls and *Pinasoy*. The result showed the most preferred was the 80:20 Pineapple/soymilk blend (3.52), while the least accepted was the 60%/40 Pineapple/soymilk on a 5-point scale (Table 1).

The taste scores for all *Pinasoy* showed no significant difference ($p > 0.05$). But the control 'A' was significantly higher ($p < 0.05$) than the others; while control 'B' (100% soymilk) was significantly lower ($p < 0.05$) than others. There was no significant difference ($p > 0.05$) in the 80%/20% pineapple/soymilk and the 70%/30% Pineapple/Soymilk. The high values attached to the 80%/20% Pineapple/Soymilk may be as a result of the higher percentage of pineapple juice which added to the sweetness and was most acceptable to the consumers (Debnath *et al.*, 2012). Akubor (2016) had reported that majority of the panellist for yoghurt and pineapple juice blend were willing to buy the blend containing 30% pineapple juice because of the pineapple juice flavour. The acceptability of the *Pinasoy* also recorded significant acceptance of the taste. Pleasant taste is the primary factor determining the acceptability of any product and has the highest impact in determining the market success of product. The overall acceptability of the *Pinasoy* decreased with increased soymilk

substitution. The overall acceptance level showed that 80%/20% Pineapple/Soymilk was the most acceptable (Table 1). The least accepted was the 60%/40% Pineapple/Soymilk. The sensory properties of *Pinasoy* decreases with an increase in soymilk in the blend. Although statistical differences were observed between control and *Pinasoy*; but the ones with up to 20% level of soymilk substitution was moderately liked. Acceptability for the 60%/40% Pineapple/Soymilk was not decisive, an indication that products of 20% and above may not have a good market. Flavourings such as vanilla have been reported to improve the high acceptability of soy beverages (Nti *et al* 2003; Villegas *et al* 2011). Sensory properties of *Pinasoy* decreased with increased soymilk in the blends, suggesting an inverse relationship between these two treatments. This is similar to the findings of Rostango *et al.* (2007). This is also consistent with those obtained in orange soy beverage formulated by Kale *et al.* (2012). They showed that, taste, aroma, colour, mouth feel and overall acceptability were preferred in the 80% orange juice, while the 10% orange juice was least accepted in the blends. Similar study (Villegas, 2009) also showed preference for soymilk with pineapple flavours to plain soy milk.

Table 1: Mean Sensory scores of Pinasoy

Sensory parameters	A	B	C	D	E	F	LSD
Aroma	3.421 ^a	2.789 ^b	3.053 ^{ab}	3.000 ^{ab}	2.947 ^{ab}	3.000 ^{ab}	0.605
Colour	4.579 ^a	3.684 ^b	3.737 ^b	3.368 ^b	3.589 ^b	3.526 ^b	0.554
Overall acceptability	4.211 ^a	2.053 ^d	3.632 ^b	3.158 ^{bc}	2.895 ^c	3.000 ^c	0.570
Taste	4.053 ^a	1.895 ^d	3.316 ^b	3.105 ^{bc}	2.737 ^{bc}	2.632 ^c	0.633
Thickness	3.632 ^a	3.579 ^a	3.526 ^a	3.421 ^a	3.000 ^a	3.105 ^a	0.684

Means with same superscript within rows are not significantly ($p > 0.05$) different

Control (A) = 100% pineapple juice; Control (B) = 100% soymilk; C= Pineapple juice (80%) + soymilk (20%); D = Pineapple juice (70%) + soymilk (30%); E = Pineapple juice (60%) + soymilk (40%); F = Pineapple juice (50%) + soymilk (50%)

Table 2: Microbial viable counts of Pinasoy

Sample	A	B	C	D	E	F
Av. Bacterial cells (Cfu/ml)	7.6×10^4	7.45×10^5	5.7×10^4	8.0×10^4	1.28×10^5	1.04×10^5
Av. Fungal cells (Cfu/ml)	1.75×10^4	1.65×10^4	3.4×10^4	2.64×10^5	3.3×10^4	2.10×10^5

Microbial status and shelf stability

The microbiological counts for the 60/40% pineapple/soymilk was 1.28×10^5 Cfu/ml for bacteria; while the 70/30% pineapple/soymilk was 2.64×10^5 Cfu/ml for fungal (Table 2). The bacteria counts were more than fungal counts. This finding is similar to the report by Ikya *et al.* (2013).

The bacteria species isolated from the *Pinasoy* treatment were *Bacillus cereus*, *Cerratia spp*, *Enterobacter aerogenes*, *E. coil*, *Bacillus spp* and *E. coil* and they were more dominant in all products except in control (Table 3a). In a similar work on microorganisms associated with locally processed milk products (Nono and Wara), *E. coli*, and *S. aureus* were isolated (Messina, 2010). *Streptococcus faecalis* was isolated in this study but absent in reports earlier mentioned. This may be attributed to the differences in environmental conditions. The presence of *E. coli* is an indication of contamination from the immediate environment similar to the findings of Liamgee *et al.* (2013) who reported the presence of *E. coil*, *Streptococcus faecalis* and *Staphylococcus aureus* in soymilk samples obtained from different locations in Benue state.

Agboke *et al.* (2011) has also reported the isolation of *E. coli*, *Staphylococcus aureus*, *Streptococcus spp* in the analysis of soymilk sold in Uyo, Nigeria. The presence of *Staphylococcus spp* in almost all the *Pinasoy* may be attributed to contamination through handling. A wide variety of spoilage microorganisms (*Staphylococcus aureus*, *Bacillus spp*, *Penicillium spp*, and *Aspergillus flavus*) were isolated (Edema *et al.*, 2004). The presence of these microorganisms is an indication of contamination of human faecal discharge and poor handling, use of contaminated raw and packaging materials, inadequate processing, unhygienic processing environment and little or no knowledge of good manufacturing practice. These gram negative microorganisms are known to be pathogenic and highly undesirable in products. Their presence may predispose the products to microbial spoilage because soymilk is a good substrate for microorganisms to grow and thus reducing microbial shelf stability.

Table 3a: Bacteria organisms isolated and their population (Cfu/ml)

Code	Population	Bacteria isolates
A	7.6×10 ⁴	<i>Bacillus cereus, Cerratiaspp</i>
B	7.45×10 ⁵	<i>Staphylococcus aureus, Bacillus aureus, Escharichia coil, Enterobacteraerogenes</i>
C	5.7×10 ⁴	<i>Bacillus cereus, Cerratiaspp, Bacillus subtilis</i>
D	8.0×10 ⁴	<i>Bacillus cereus, E. coil, S. aureus</i>
E	1.28×10 ⁵	<i>Bacillus subtilis, Enterobacteraerogenes</i>
F	1.04×10 ⁵	<i>Bacillus spp, E. coil, Cerratia spp</i>

Table 3b: Fungi organisms isolated and their population (Cfu/ml)

Code	Population	Fungi isolates
A	1.75 × 10 ⁴	<i>Aspergillusniger, Aspergillus flavus</i>
B	1.65 × 10 ⁴	<i>Aspergillus flavus, Fusarium spp, Penicillium spp</i>
C	3.4 × 10 ⁴	<i>Aspergillus flavus, Penicillium spp</i>
D	2.64 × 10 ⁵	<i>Aspergillus flavus, Penicilliumcrusatum</i>
E	3.3 × 10 ⁴	<i>Penicillium spp, P. digitarium</i>
F	2.10 × 10 ⁵	<i>Penicillium spp</i>

Table 4: Microbiological status (characteristics) of Pinasoy at room temperature for a period of seven (7) days

Days	Sample	Appearance	Taste	Aroma
1 – 3	A	Yellowish	Pleasant	Pleasant
	B	Milky	Unpleasant	Beany
	C	Yellowish-milky	Pleasant	Pleasant
	D	Yellowish-milky	Pleasant	Pleasant
	E	Yellowish-milky	Pleasant	Creamy
	F	Milky	Pleasant	Creamy
4 – 7	A	Yellowish	Sour	Sharp
	B	Milky	Bitter	Off-soy flavour
	C	Yellowish-milky	Sour	Slightly sharp
	D	Yellowish-milky	Sour	Slightly sharp
	E	Yellowish-milky	Sour	Slightly sharp
	F	Milky	Sour	Slightly sharp with off-soy flavour

Some of the fungal species isolated were *Aspergillus spp, Fusarium spp, Penicillium spp. Aspergillus spp* were more present in the products except in the 60%/40% Pineapple/Soy milk and 50%/50% Pineapple/Soy milk in which *Penicillium spp* was implicated; the populations being 3.3 × 10⁴ and 2.10 × 10⁵ Cfu/ml (Table 3b). The presence of *Aspergillus spp* in this study is in agreement with the findings of Agboke *et al.* (2011). The presence of *Aspergillus spp* and *Penicillium spp* may result in *mycotoxicosis* which may pose serious health problems to consumers. *Aspergillus spp* have also been implicated in causing *mycetoma* in human (Cheesbrough, 2000). *A. flavus* involved in allergic *aspergillosis (pulmonary aspergillosis)* and also produces aflatoxin that is highly carcinogenic (Prescott *et al.*, 2005). Iloju and Iloh (2007) isolated and identified *A. flavus* and *A. niger* from sorrel drink. It is worthy of note however, that these organisms isolated, were present in population far less than the lower threshold (>10⁶) for *delicatessens* (ready-to-eat foods) as set by FAO (2008). In addition, the presence of these microbes may not be harmful to consumers as some of them assist in the enzymatic breakdown of food and some synthesize useful vitamins. The prolonged storage of these microbes can bring about the microbial spoilage of the beverage. Pelczar (1986) had earlier documented that *Bacillus aureus* is among the microorganisms responsible for the spoilage of *tofu* (a soymilk product). This also is in agreement with the report of Ogbulie (2001), who noted that non-pathogenic genera of microorganisms such as *Streptococcus, Lactobacillus* and *Bacillus aureus* survived pasteurization and eventually spoiled milk product. The

characteristics changes in physical forms e.g. colour, taste, aroma and flavour over time in storage at room temperature are recorded in Table 4.

Conclusion

Apart from pineapple, some other fruits juice be incorporated into the soymilk. However, it is important to evaluate the chemical composition and sensory quality of newly developed products in order to ascertain acceptability by consumers. In this current work, it has been shown that soymilk with pineapple juice are very nutritious to the human. The treatment sample C and D had similar nutritional and sensory characteristics with the control A and can be generally accepted by the public. The use of this up to 20% level of the soymilk substitution would help improve the use of domestic fruits (such as pineapples, orange etc) for soymilk production. This study has shown that the most accepted was the 80/20% pineapple/soyamilk blend for the *pinasoy* drink followed by the sample D 70/30% Pineapple/Soyamilk blend. The pineapple juice was used to mask the undesirable “beany” flavour of soymilk. The acidic nature of the pineapple juice makes it hard for some microorganism to survive in the Pinasoy. The microbial population estimated by the total aerobic count in the samples was lower than the acceptable standard and this poses no serious health hazard to consumers. Considering the resistance of *Staphylococcus aureus* to *Penicillin, methicillin*, its detection regularly in the Pinasoy sample analysed poses a serious health hazard to the consumers but if the soybean and pineapple juice are processed in a sterilized unit and well preserved through refrigeration in order to reduce microbial growth, this will hinder such problem.

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