

Full Length Research Paper

Microbiological analysis of flavoured yoghurts

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Production and quality evaluation of plain yoghurt, spiced yoghurt (pepper fruit, ginger) and flavoured yoghurt (carrot and pineapple) were carried out being proximate composition, mineral analysis, microbiological analysis, organoleptic evaluation and statistical analysis. Results show significant ($p < 0.05$) nutritional enhancement of the plain yoghurt by the addition of spices and flavourings. The mineral content of the plain yoghurt were likewise increased. Organoleptically, the spiced and flavoured yoghurts were all acceptable by consumers but pepper fruit spiced yoghurt was the most preferred in terms of general acceptability.

Key words: Yoghurt, pepper fruit, carrot and pineapple.

INTRODUCTION

Yoghurt is a fermented dairy product obtained from the lactic acid fermentation of milk. It is one of the most popular fermented milk products in the world (Willey et al., 2008).

Bourlioux and Pochart (1988) defined yoghurt as a coagulated milk product that results from the fermentation of lactose in milk by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Other lactic acid bacteria (LAB) are also frequently used to produce yoghurt with a unique characteristic (Adolfsson et al., 2004). It is a nutritionally beneficial product generally considered safe, with taste liked by many people.

Yoghurt is produced commercially by pasteurising the milk mixture, cooling to 45°C before being inoculated with

known cultures of microorganisms referred to as starter culture. The starter culture may be mixed *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in a ratio of 1:1. They act on lactose and result in the production of lactic acid which increases the acidity of the yoghurt, thereby forming gel.

The decrease in pH inhibits the growth of pathogenic bacteria. The lactic acid produced is also responsible for the characteristic flavour and aroma of yoghurt and helps to maintain the quality of the yoghurt during storage and packaging (Saint et al., 2006).

Yoghurt can boost immunity. The regular consumption of live cultured yoghurt produces a higher level of immunity boosting interferon as this bacteria cultures stimulate infection-fighting white cells in the blood stream with anti tumor effects (Maltock, 2007). Yoghurt is nutritionally rich in protein, carbohydrate, vitamins and minerals (for example calcium) which contributes to a

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healthy living including decreasing the risk of colon cancer, improved digestion and many other benefits (Gray, 2007).

Sensory appeal is one of the essential strategies associated with the market success of fermented products like yoghurt. The popularity of yoghurt as a food component has been linked to its sensory characteristics (Routray and Mishra, 2011). Furthermore, there is need to introduce other fruit and vegetable flavours like carrots, pineapple and spices such as ginger and pepper fruit so as to produce yoghurt with spicy taste with a characteristic aroma and fragrance. Also, to improve the nutritional composition of yoghurt produced.

Health benefits of yoghurt

Lactobacillus, a probiotic (friendly) bacteria found in yoghurt offers remarkable preventive and curative effects on arthritis (Gray, 2007). Yoghurt is easier to digest than milk and so many people including children who cannot tolerate milk, either because of a protein allergy or lactose intolerance can enjoy yoghurt more digestible than milk. Bacterial enzymes created by the culturing process partly digest the milk protein casein making it easier to absorb and less allergenic (Witton, 2004). Yoghurt contains intestine friendly bacteria culture (lactobacteria) that fosters a healthy colon and lowers the risk of colon cancer by promoting the growth of healthy bacteria and thereby deactivate harmful substances which can cause problem in the colon (Gray, 2007). It is also rich in calcium which contributes colon health and decreases the risk of colon cancer (Gray, 2007).

The regular consumption of live cultured yoghurt produces a higher level of immunity boosting interferon as these bacteria cultures stimulate infection fighting white cells in the blood stream with anti tumor effects (Maltock, 2007). Daily consumption of ounces (100 g) of yoghurt significantly improved the cholesterol while raising high density lipoprotein (HDL) (good cholesterol). This may be because of the ability of the live culture in yoghurt to assimilate cholesterol or because yoghurt binds the bile acids which lowers cholesterol (Maltock, 2007).

Carrot (*Daucus carita*)

Carrot (*Daucus carita*) is a root vegetable, horn like in shape, usually orange in colour, though purple, red, white, and yellowed varieties exist. It has a crisp texture when fresh.

The most commonly eaten part of the carrot is taproot, although the green are sometimes eaten as well. It is a domesticated form of the wild carrot *D. carota*, native to Europe and South Western Asia. The domestic carrot has been selectively bred for its greatly enlarged and

more palatable, less woody textured edible taproot. Carrots are widely used in many cuisines, especially in the preparation of salads, and carrot salads are traditional in many regional cuisines (Mabey, 1997; Rose, 2006).

Pineapple (*Ananas comosus*)

A. comosus is a tropical plant in the Bromeliaceae family. This delicious fruit is full of nutrients that promote good health. Raw pineapple are loaded with vitamins, enzymes and minerals including vitamin A, vitamin C, calcium, phosphorus, manganese and potassium, which are important to health, it is also rich in fibre and calories and low in fat and cholesterol (Marcela, 2012). Both the root and fruit may be eaten or applied topically as an anti-inflammatory. The anti-inflammatory properties can greatly alleviate the pain of arthritis (Marcela, 2012).

Pepper fruit (*Dennettia tripetala*)

Pepper fruit (*D. tripetala*) is a well-known forest fruit and indigenous spicy medicinal plant. It is a tropical rain forest plant widely domesticated in the Southern, Eastern and Western parts of Nigeria (Chandraseharen, 1994). It can be chewed in different forms. Okafor (1980) reported that pepper fruit (*D. tripetala*) contains minerals and vitamins. Pepper fruit is a spice medicinal plant for curing fever, cough, toothache, as well as stimulant and is used in the preparation of some special dishes for pregnant and postpartum women, during which it is claimed that the spices and herbs aid in uterine contraction (Oyemitan et al., 2006).

Ginger (*Zingiber officinale*)

Ginger (*Z. officinale*) is consumed as a delicacy, medicine or spice. Ginger produces a hot, fragrant kitchen spice. Young ginger rhizomes are juicy and fleshy with a very mild taste. They are often pickled in vinegar or sherry as a snack or just cooked as an ingredient in many dishes. They can also be steeped in boiling water to make ginger tea. Ginger is generally prized for its use as a herb, flavour and as a spice, not as a nutritional supplement. According to Catherine (2010), 2 g of ginger contains only 1.6 calories, 0.7 mg of omega-3 fatty acids and 2.4 mg of omega-6 fatty acids. It provides 0.1 mg of vitamin C and 0.2 mg of folate. Ginger also contains minute amounts of minerals: calcium (0.3 mg), magnesium (0.9 mg), phosphorus (0.17 mg), potassium (8.3 mg) and sodium (0.3 mg).

This work will introduce new varieties of flavoured and spiced yoghurt from carrot (*D. carita*), pineapple (*A. comosus*), pepper fruit (*D. tripetala*) and ginger (*Z. officinale*),

which will be of good nutritional quality and health benefits. This work will also reveal and get people informed of the nutritional significance and additional usefulness of these fruits and spices; thus increasing their importance to mankind.

The objectives of this research included production of flavoured and spiced yoghurt using ginger, pepper fruit, carrot and pineapple; evaluation of nutritional quality and the flavoured and spiced yoghurts; determination of microbial load of the flavoured and spiced yoghurt and determination of organoleptic qualities of the flavoured and spiced yoghurts.

Ingredients for yogurt production

Milk

Milk is the major product in the production of yogurt (Lopez, 1997). The type of milk used depends on the type of yogurt: whole cream milk for full fat yogurt, low fat milk for low fat yogurt. Milk in yogurt a rich flavour and smooth texture, contribute to energy and forms cream formation with water.

Starter culture

The main starter culture used in yogurt production is *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. They can grow independently but the rate of acid production is much higher when used together than when used individually.

S. thermophilus grows faster and produces both acid and carbon dioxide. The production of carbon dioxide stimulates the growth of *L. bulgaricus*. On the other hand, the proteolytic activity of *L. bulgaricus* produces stimulatory peptides and amino acids used by *S. thermophilus*. These micro-organisms are ultimately responsible for the formation of typical yogurt flavour and texture. The yogurt mixture coagulates during fermentation due to drop in pH. The streptococci are responsible for the initial pH drop of the yogurt mix to approximately 5.0. The Lactobacilli are responsible for the further decrease of pH to 4.5.

Gelatin

Gelatin is a protein produced by partial hydrolysis of collagen extracted from boiled bones, connective tissues, organs and some intestines of animals such as domesticated cattle, pigs, and horses. It is used as a stabilizer to increase the firmness and viscosity of yogurt. Tamime and Robinson (1999) reported that gelatin has the ability to bind water, reacts with the milk constituents (mainly proteins), and stabilize the protein network preventing free movement of water. Gelatin tends to degrade during process at high temperature.

Consequently, the yoghurt gel is considered weakened. Gelatin is used at a level of 0.3-0.5% to get a smooth shiny appearance and should be geared to the consistency standard for yoghurt. Alais and Linden (1999), disclosed that gelatin has a wide spread uses in foods without any limits imposed.

Water

Water plays an important role in yogurt making. The quantity of water must be carefully controlled such that the milk and the bacteria have correct available amount of water to make a yogurt of proper consistency.

Water provides the following functions in the making of yogurt: It creates an enabling environment for multiplication; it helps in the homogenization and forms the medium for enzyme activation and it helps to control yogurt temperature.

MATERIALS AND METHODS

Collection of samples

Skimmed powdered milk, carrot, pineapple, pepper fruit, ginger, gelatin, yoghurt culture and granulated sugar produced by Dangote Sugar Company were obtained from Eke-Ukwu Market in Owerri municipal council of Imo State, Nigeria.

The practical work which involved the production of flavoured and spiced yoghurt was conducted in the Food Processing Laboratory of the Department of Food Science and Technology, Imo State University, Owerri, Imo State. The laboratory analysis of the yoghurt samples was carried out at National Root Crop and Research Institute, Umudike in Abia State.

Sample processing

The samples of yoghurt were produced according to International Standard of yoghurt as described by Guler and Mutlu (2005). Here 400 g of skimmed powder milk was reconstituted with water and heated to 80°C for 15 min for pasteurization, and then allowed to cool to 42-45°C before inoculation with starter culture. The milk mixture was divided into five portions, raw yoghurt, carrot flavoured (sample C), pineapple flavoured (sample B), pepper fruit spiced (sample D), ginger spiced (sample E). They were incubated at 43°C for 10-12 h (overnight) until a pH of about 4.3-4.5 was attained. The yoghurts were cooled at 6°C in a refrigerator before they were analyzed.

Proximate analysis

The total solid of the yoghurt sample was determined using gravimetric method described by AOAC (1999).

The moisture and ash content of the yoghurt samples was determined using the indirect method employing drying oven and furnace incineration method described by Onwuka (2005). The protein content of the sample was determined by the semi-micro Kjeldahl, method reported by AOAC (1990).

The fat content of the sample was determined on wet weight basis by Soxhlet's method as described by Suzanne (2003). The carbohydrate content of the sample was determined by estimation using the arithmetic difference method described by James (1995).

Mineral determination

The resulting ash was dissolved in 100 ml of dilute hydrochloric acid (HCL) and then diluted to 100 ml in volumetric flask using distilled water. The digest so obtained was used for the mineral analysis.

Phosphorus in the sample was determined by the vanado-molybdate (yellow) spectrometry described by James (1995). Calcium and magnesium contents of the test samples was determined by the ethylenediamine tetraacetic acid (EDTA) complexometric titration of AOAC (1990).

Potassium content of the sample was determined by flame photometry. The instrument was set up according to the manufacturer's instruction. The equipment was switched on and allowed to stay for about 10 min. The gas and air lets were opened and the start knob was turned on AOAC (1990).

Microbiological analysis of samples

Determination of microbial load (coliform, bacteria and fungi load)

The method of the international commission on microbiological specification for foods ICMSF (1978) was adopted and used.

Total viable microbial count

A suspension of the bacteria was serially diluted and aliquots of each dilution was placed in suitable culture media (Hausler, 2003).

Organoleptic evaluation

The product samples were evaluated using hedonic method for sensory characteristics and overall acceptability by a panel of 40 judges selected randomly. They were served coded samples of yoghurt and asked to compare it by testing for taste, aroma, texture, appearance, and overall acceptability. All tests were performed and rated on a 9-point hedonic scale described by Ihekoronye and Ngoddy (1985) : like extremely, 9; like very much, 8; like moderately, 7; like; slightly, 6; neither like or dislike, 5; dislike slightly, 4; dislike moderately, 3; dislike very much, 2; dislike extremely, 1.

Statistical analysis

The data obtained from sensory evaluation was analysed using analysis of variance (ANOVA), according to the method of Iwe (2002) to determine the variance ratio. Sample means were compared to determine treatment effects. The least significant difference was calculated at 95% level of significance using tukey test (T-test), (Ihekoronye and Ngoddy, 1985).

RESULTS AND DISCUSSION

Proximate composition of plain

Yoghurt

The proximate composition of plain yoghurt (neither flavoured nor spiced) shows protein (9.97%), moisture (84.67%), fat (1.80%), Ash (0.44%), and carbohydrate

Table 1. Nutritional composition of plain yoghurt (PY).

<u>Parameter</u>	<u>Plain yoghurt</u>
CHO	1.70
Protein	9.97
Fat	1.8
Ash	0.44
Fibre	0.32
Moisture	84.67

Table 2. Vitamin (mg) of yoghurt samples composition.

<u>Vitamins</u>	<u>PY</u>	<u>PFY</u>	<u>GFY</u>	<u>CFY</u>	<u>PFY</u>
Vitamins A	5.87	6.05	5.86	6.66	5.92
Vitamins C	3.90	4.01	3.91	4.25	4.48
Riboflavin	0.52	0.53	0.53	1.00	0.89
Thiamine	1.86	2.57	1.86	3.90	1.92
Niacin	2.01	2.02	2.01	2.02	2.25

(1.70%) contain (Table 1).

The high moisture content of the product could be as a result of the dilution (reconstitution) of the milk prior to fermentation. The low fat content of the yoghurt could be attributed to the low oil content of the milk (Skimmed milk) which was the major substrate of the yoghurt produced.

This corresponds with the work of Amna et al. (2008) that non-fat (zero%) yoghurt can be produced but in general, the fat level of every yoghurt depends on oil content of the milk, whether skimmed or full cream milk. He stated categorically that yoghurt manufactured from skimmed milk will likely have very low fat content (within the range of 1-2%) while those produced from full cream milk will have fat content in the region of 4% (or slightly above).

Also the ash and fibre content were remarkably low and this result agrees with observation of Cheeseman and Lean (2000) that generally, yoghurts have poor fibre level because they are milk and water based products.

Carbohydrate (lactose) is the major constituents of milk that is converted to lactic acid during yoghurt (fermentation) production task of the yoghurt. So the fermentation and conversion of lactose to lactic acid accounts for the low content of carbohydrate of yoghurt as observed in the result (Table 2). This corroborates with the works of Mistry and Hassan (1992) and Younus et al. (2002)

The observed protein content (9.97) of the plain yoghurt compares favourably with commercial standard stated by National yoghurt Association (2000), that commercial yoghurt should have 11-18% protein. Also, Adolfsson et al. (2004) reported protein content (1003) which is in agreement with the result in Table 1.

Table 3. Mineral analysis of yoghurt samples.

Parameter	PY (mg/g)	PFSY (mg/g)	GSY mg/g	C FY mg/g	PFY mg/g
Calcium	180	180.05	180.00	180.00	180.00
Phosphorus	158	158.12	158.33	158.04	158.10
Magnesium	170	170.08	170.001	170.14	170.00
Sodium	111	111.06	111.10	111.07	111.10
Potassium	121	121.50	121.02	121.11	121.12
Iron	108	110	108	108.1	108.1

Mineral analysis of raw plain, flavoured and spiced yoghurts

Mineral analysis of plain yoghurt (mg/g) revealed calcium (180), phosphorus (158), magnesium (170), sodium (111) and potassium (121) content. The result justifies the ascertainment of Gray (2007) that yoghurt is a very good source of essential minerals needed for human metabolism or functionality of cells. Addition of pepper fruit and carrot respectively caused slight increase in calcium, phosphorus, magnesium, sodium and potassium (0.5, 0.11). A similar increase in minerals was also observed by Ihemeje et al. (2013) where pepper fruit was used in Zobo drink production.

D. tripetala (seed) has earlier been reported to contain minerals, vitamins, and oils (Okafor, 1980), protein, fibre, ash, and carbohydrate (Udoessian and Ikon, 1984; Okwu et al., (2004). Carrot according to Cohen et al. (2010) is rich in calcium, magnesium and potassium. This may have caused the observed increase in mineral value of the products. Carrot is well known for its B- carotene content (a precursor of vitamin A) and fruits containing B carotene can be used in the management of vibroacoustic disease (VAD) in adults especially in poor resource countries (Novotry et al., 1995)

The iron content was only remarkably improved in sample B (pepper fruit flavoured). This further justifies that pepper fruit contains appreciable quantity of iron as earlier reported by Ihemeje et al. (2013)

Ginger and pineapple respectively caused varied increase phosphorus (0.03,.11), sodium (0.1,0.1) and potassium (0.02, 0.12). Ginger is known for its pungent and stimulant effects and contains more oleoresins than minerals (Connell, 1970; Nwinuka et al., 2005). It has more of Cherepeutic effects than nutritional. On the other hand, pineapple contains more vitamins and minerals than ginger. (Nutrient data pineapple, 2012) especially water soluble Vitamins (Vit. C.) This explains why higher value of minerals was observed in pineapple favoured yoghurt than ginger spiced yoghurt.

Vitamin contents of plain flavoured and spiced yoghurt

The vitamin contents of plain flavoured and spiced yoghurts

Table 4. pH value of the yoghurt samples.

Yoghurt sample	pH
Plain	4.6
Pepper fruit spiced	4.7
Ginger spiced	4.7
Carrot flavoured	4.7
Pineapple flavoured	4.5

Value are means of triplicate determination.

is shown in Table 4. The plain yoghurt contained vitamin A (5.87 mg), vitamin C (3.90 mg) riboflavin (0.52 mg), thiamine (1.86 mg), niacin (2.01). Addition of pepper fruit, carrot and pineapple respectively caused improvement in vitamin A (0.18, 0.79 and 0.05), vitamin C (0.11, 0.35 and 0.58), riboflavin (0.01, 0.48 and 0.37), thiamin (0.71, 2.04 and 0.06) and niacin (0.01, 0.01 and 0.24) contents above that the observed increased in the vitamin contents could be a justification of the report that carrot (Cohen et al., 2002) pepper fruit (Ihemeje et al., 2013) and pineapple(Hale et al; 2010) are all rich in vitamin .similar trend of increase in vitamin content of flavoured and spiced yoghurts was respec-tively recorded by Amna et al. (2008) and Mbaeyi and Anyanwu(2010)

The pH of the various yoghurt sample are presented in Table 3 result indicates that pineapple flavoured by the plain yoghurt (4.6) pepper fruit spiced yoghurt, ginger spiced yoghurt and carrot flavoured yoghurt had the same pH (4.7). Addition of pineapple as a flavourant caused the pH to drop from 4.6 to 4.5. This could be attributed to two reasons; the pineapple juice contains appreciable quantity of ascorbic acid, also it was not diluted with water.

Unlike ginger, carrot and pepper fruit that were extracted with water before adding them into the yoghurt. A corresponding alteration of pH of plain yoghurt was observed by Gabriel et al. (2013) in their work on the production of probiotic yoghurt flavoured with the spice *Aframomum danielli*, strawbeing and vanilla. The result also corroborates earlier research by Mbaeyi and Anyanwu (2010) on production and evaluation of yoghurt flavoured with solar dried bush mango.

Table 5. Microbial count of plain, flavoured and spiced yoghurt.

Simple	Dilution factor	Total bacterial count (cfu/ml)	Total coliform (MPN/100 ml)
PLY	10 ²	0.8×10 ³	0
PFSY	10 ²	1.0×10 ³	1
GSY	10 ²	0.9×10 ³	0
CFY	10 ²	1.4×10 ³	1
PFY	10 ²	1.3×10 ³	1

PLY, plain yoghurt; PFSY, pepper fruit spiced yoghurt; GSY, ginger spiced yoghurt; CFY, carrot flavoured yoghurt; PFY, pineapple flavoured yoghurt.

Microbiological assessment of plain yoghurt

Coliform group of bacteria

The Table 5 shows the results obtained after microbial examination of the samples. There was no evidence of coliform in samples PLY and GSY while PFSY, CFY and PFY had some evidence of coliform after production. That could be because of the extraction of pineapple, pepper fruit, ginger and carrot during processing.

Turkish standard institute, (1989), state that a maximum count of 10 cfu/ml of coliform group bacteria was allowed in yoghurt. So, the samples with values less than 10 cfu/ml are therefore justified suitable and safe for consumption. But absence of coliform will help extend the shelf-life of the products.

Different studies showed higher yoghurt counts of the coliform group bacteria in yoghurt samples. Dun and Ozgunes (1981) reported that 30 cfu/ml of coliform group bacteria was found in the yoghurts sold commercially in Ankara, Turkey.

Total bacteria count

The total bacterial count of the microbiological analysis showed sample PFY had (0.8X10³) PFSY had (1.0X10³) GSY had (0.9X10³) CFY had (1.410³) PFY had (1.3X10³).

There was no much difference in the total bacterial count in the spiced and flavoured yoghurts.

Micro-organisms used as starter culture have contributed to the yoghurt total bacterial count of the yoghurt samples.

Also the source (farm to market) of flavourants (pineapple and carrot) and spice (pepper fruit and ginger) may have contributed to the total bacterial count which is above that of plain yoghurts.

The bacterial count levels are very much within or below the acceptable range (8.7cfu) according to National Yoghurt Association (NYA, 2006).

Conclusion

From the results obtained, the nutritional quality of the

plain yoghurt was improved by the addition of pepper fruit, ginger, carrot and pineapple especially in terms of vitamins and minerals. This implies that the therapeutic potency of yoghurt could be improved because consumption of foods with high vitamins A and C can aid in combating deficiency diseases like scurvy and night blindness. Adequate supply of minerals improve functionality of cells and also supports immunity of the body. The sensory attributes (colour, texture, aroma, taste, general acceptability) evaluated revealed appreciable degrees of acceptance by consumers thereby increasing varieties of yoghurts, in the market. This also justifies additional economic importance /use of pepper fruit, ginger, carrot and pineapple. Pepper fruit spiced yoghurt was most preferred in terms of general acceptability.

Recommendation

It is recommended that information on the production of spiced and flavoured yoghurts using pepper fruit, ginger, carrot and pineapple should be disseminated to domestic and commercial manufacturers of yoghurts and also it very necessary that further work should be done where pepper fruit, ginger, carrot and pineapple may be incorporated in yoghurt formulation before fermentation rather than being added as mere flavourants or spices. This research if carried out would reveal nutritional implication and organoleptic attributes of such products.

Conflict of interests

The authors did not declare any conflict of interest.

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