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Full Length Research Paper

Phytochemical screening and application of extracts of selected plant foods in preparation of enhanced sensorial and healthier image yoghurt

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Extracts of four indigenous plant foods namely: *Chrysophyllum albidum* (*Cal*), *Curcuma longa* (*Clo*), *Tetracapidium conophorum* (*Tco*) and *Piper guineese* (*Pgu*) were screened for phytochemical endowment in order to gain insight to their pharmacological potentials with concomitant propensity for pro-lactic acid fermentation. The extracts were used in the formulation of substrate for preparation of modified yoghurt. Physicochemical and sensory properties of the modified yogurt were assessed in comparison to plain (unmodified) yogurt. Assessment revealed the presence of phytochemicals of therapeutic importance with pro-fermentation values principally, flavonoids, saponins, sugars and peptides in *Clo* and *Cal*. Alkaloids dominant extract exhibited anti-fermentation effects. Modified yoghurt preparations using *Clo* and *Cal* with respect to water (in place of extract) were characterized with improved quality. Titratable acidity ($x10^{-2}$ g/ml); 140, 50, 45 and relative reducing power ($x10^{-3}$ g/ml) of 9.53, 9.56, 1.38 were found for *Clo* – plain yoghurt (YOG) and YOG respectively. Comparatively to YOG, the extracts conferred assorted sensorial (P = 0.05) qualities with *Clo* - YOG most preferred. The extracts of the indigenous plant foods can be used to enhance the physical and healthier image of yoghurt.

Key words: Plant foods extract, phytochemicals, yoghurt, physicochemical properties, healthier image, sensorial characteristics.

INTRODUCTION

Yoghurt is a fermented milk product with probiotics functionality widely consumed around the world. Sodim et al. (2005) reported that in 2001, more than nine million tons of yoghurt were produced. Similarly, popularity and acceptable of yoghurt is on the increase due to its health benefits, which could be in terms of its beneficial microflora from the lactic acid fermentation, and also, yoghurt has reduced lactose level and active enzymes which may allow lactose intolerant individual to consume moderate amounts of the diary food (Posecion et al., 2005). Yoghurt properties essentially are influenced by three principal factors. They are substrate composition (bulk and additive), inoculums characteristic and processing conditions. Among the factors, use of additives is the simplest but effective means to influence yoghurt property. Additives can be synthetic or natural in origin. The notion that recovery of harmony between man and nature with respect to food and nutrition as the cheapest and effective strategy to public health management advocates preference for application of additives of natural origin instead of the use of synthetic additives that have been implicated with health risks. Majority of natural additives are parts (leaf, seed, fruit, root etc) or extracts of plants. The plant extracts should enhance the functionality of the food by addition during processing, other than mere addition to the finished product. Thus, influences the sensory and chemical characteristics of such food as demonstrated by

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Mohammadi et al. (2012) during preparation of yoghurt. In addition, plant extract from lemon grass has been used as food additive in preparation of yoghurt (Abd El Fattah et al., 2010). Also, agricultural substrates have been studied as alternative low cost substrates for microbial fermentation (Rodrigues et al., 2007).

Library of information exist in literature (Trease and Evans, 2002) on health or physiological importance of bioactive components of plants (foods and non-foods) that have long history of safety. The major biochemical conditions supporting the survival of fermentation organisms in cultured diary processes essentially are: reductones, metabolizable substrates in nature of sugars and peptides. Therefore, plant food(s), rich in sugars and peptides, with bioactive components that possess reducing activity might exhibit pro-fermentation activity thus deserve investigation for use in production of yoghurt with healthier food potentials. However, despite the potentials of plant extract as low cost substrate and additive, their complex composition may have some substances that may interfere with fermentation, hence it is necessary to ascertain the effect of some plant food extracts on preparation of yoghurt.

In this study, extracts of four different plant foods with long history of safety namely *Curcuma longa, Tetracapidium conophorum, Chrysophyllum albidum* and *Piper guineese* were examined for phytochemical endowment and influence on yoghurt preparation with respect to physicochemical, sensory and selected health promotion induces.

MATERIALS AND METHODS

The materials used included powder whole milk (peak milk), a product of WAMCO Nigeria, skimmed milk, and sugar. The plant foods *C. longa* (rhizome), *T. conophorum* (seed), *C. albidum* (fleshy pulp) and *P. guineese* (seed) were obtained from the local market and starter culture was obtained from commercial food store.

Starter culture

The starter culture used was blends of strains of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* with the trade name yoghurtmet culture (de yoghurt sechee A. Froid, Lyo – San Inc., 500 Aeroplane C. P. 598, Lachute (QC) Canada JBH 4G4. The starter culture was stored at -70°C in its packet prior to application.

Yoghurt preparation

Yoghurt was essentially prepared according to the method described by Sodim et al. (2005). Simply, milk was standardized to a fat level (10 g Kg^{-1}) by blending pasteurized non-fat milk and homogenized whole milk. The protein content (45 g Kg^{-1}) was enriched by additions of skimmed milk powder. 1 L flasks were filled with the standardized and fortified milk, heated at 9°C, prior to inoculation (0.2 g l^{-1}) of the commercial starter culture. Then, incubated (28 ± 2°C) for 16 h. Fermentation was stopped by rapid cooling at 2°C in a refrigerator.

Application of plant foods extract

Each of the plant foods (2 g) namely: *C. longa, T. conophorum, C. albidum* and *P. guineese* was size reduced and extracted using water (60 ml) at a temperature of 90°C for 20 min (excluding come-up time). The aliquot was filtered and concentrated by heating at same temperature for another 15 min to obtained *Clo, Tco Cal* and *Pgu* extracts respectively of the afore-mentioned plant foods. The extracts were added (15% $^{v}/_{v}$) to standardized milk prior to addition of starter culture. Water was added to substrate (milk) in place of extract for the control sample. The preparation were subsequently fermented to obtain *Clo*-YOG, *Tco*-YOG, *Cal*-YOG, *Pgu*-YOG (modified yoghurt preparations) and YOG (unmodified or plain yoghurt) for *Clo, Tco, Cal, Pgu* extracts and water added (YOG) samples, respectively.

Phytochemical screening

Phytochemical investigations on extracts were performed as described by Trease and Evans (2002), and Harborne (1984). Meyers reagent was used for alkaloids, Molish test for glycosides, the Biuret reagent for peptides, Mg-HCl reagent for flavonoids, Libermann-Buchard reagents for steroids and frothing method was for detection of saponins.

Determination of titratable acidity

Separately, plant extracts and yoghurt preparations were titrated with standard alkaline reagent. Results were expressed as lactic acid equivalent (AOAC, 1984).

Measurement of pH

The pH of samples was measured using Omega H. HP_x digital pH meter. Standardization of the meter was done using buffer solutions of pH 4 and 9.

Evaluation of total phenolic content

Total phenolic content was evaluated according to the method described by Taga et al. (1984). Briefly, a 100 µL of Folin-Ciocalteau reagent (2N wrt acid Fluka Chemic AG-Ch-9470 BUCHS) was added to each sample (20 µL) and well mixed after addition of 1.58 mL of water. After 30 s, 300 µL of 2% sodium carbonate solution was added and the sample tubes were left at room temperature for 2 h. The absorbance (A) of the developed blue colour was measured at 750 nm using Unicam Helios and ultraviolet-visible) (UV-VIS) spectrophotometer. A plot of A_{750nm} against corresponding concentration was used to calculate phenolic content (g/g ascorbic acid equivalent).

Determination of relative power

Reducing power of each sample was determined in accordance with the method of Oyaizu (1986). Simply, each sample (1 mg/mL) in methanol (2.5 mL) was mixed with sodium phosphate buffer (pH 6.6). The buffered sample was mixed with conditioning reagents (1%K_3-Fe-CN_6, 10% TCA, 0.1% $FeCl_3$), centrifuged, diluted using distilled water and absorbance was measured at 700 nm. Higher absorbance indicates a higher reducing power.

Water holding capacity (WHC)

WHC was determined according to a procedure described by

Table 1. Phytochemical characteristics of the selected plant food extracts.

Phytochemical component	Sample			
	Clo	***Tco***	***Cal***	***Pgu***
Alkaloids	-	+	-	
Peptides	++	-	+++	-
Reducing Sugar	++	-	+++	+
Flavonoids	+	-	-	+
Glycosides	+	+	++	+
Steroids	+	-	-	++
Saponins	+	-	+	-

Clo, Water extract of *Curcuma longa; Tco*, water extract of *Tetracapidium conophorum; Cal,* water extract of *Chrysophyllum albidum; Pgu,* water extract of *Piper guineese;* ++, test positive; +, slightly test positive; -, not detected.

Sodim et al. (2005) with slight modification. A sample (modified or plain) of 20 g of yoghurt (Y) was centrifuged for 10 min at 1250 x *g* at 4°C; the whey (W) expelled was removed and weighed. The water holding capacity (WHC) was calculated as:

$$WHC\ (\%) = \frac{Y-W \times 100}{Y}$$

Where, Y = weight of yoghurt and W = weight of whey.

Relative viscosity (ηrel)

ηrel was evaluated using Baroid division rheometer according to the method described by Myers and Smith (1964). Water was used as reference.

Sensorial evaluation

Using multiple comparison test, sensorial evaluation of the different yoghurt (modified and plain) was carried out by eight trained panellists that comprised of students of the Department of Food Technology, Federal Polytechnic, Ado-Ekiti. Sensorial attributes evaluated were taste, mouth feels, colour, odour, using a score scale of 1 to 7 where 7 indicates extremely like and 1 indicates extremely dislike (Larmond, 1979).

Statistical analysis

All data were measured in triplicate (except sensorial score data) and subsequently analyzed using a one-way analysis of variance and Newman-Keuls multiple comparison test (Prims ^{R}, Graph Pad, San Diego, CA). Values of P = 0.05 were considered significant, if otherwise, it was stated.

RESULTS AND DISCUSSION

Phytochemical characteristics of the selected plant food extracts

Even though the selected samples are plant foods, it is lore that they are of immense therapeutic value in folk medicine. Similarly, medicinal applications of all the samples had been stabled (Adeniji, 2003; Hollist, 2004) in traditional medicine in Nigeria. Therefore, it is important to screen them in order to gain information regarding their phytochemical endowment, because bioactive constituents have positive relationship with respect to health benefits if ingested. The phytochemical characteristics can also inform on pro-fermentation potentials of the bioactive components in the plant foods.

The result of the preliminary phytochemical investigation using water extracts of the plant foods (*Clo, Tco, Cal* and *Pgu)* is shown in Table 1. Assessment showed that alkaloids tested positive in *Tco* and *Pgu.* The result is in agreement with the data published on bioactive components of *T. conophorum* as reported in Medicinal plants of Nigeria (NNMDA, 2006). Peptides and sugars were detected at high degree in *Cal* and *Clo.*

In supports of the observation, non enzymatic browning that dominated *Cal* could be as a result of glycation products of peptide and sugar that developed during high temperature of drying process and shortage. Peptides and sugars are substrates capable of supplying two nutrient factors namely nitrogen and carbon sources required for fermentation.

Flavonoids were detected in *Clo* and *Pgu.* Of all the polyphenolic compounds, flavonoids have been the major class of phytochemical component characterized with antioxidative activity (Foti and Ruberto, 2001). All the samples showed test positive for glycosides. Considering the structural complexity and relationship of saponins and steroids as well as the limitation of phytochemical screen methods employed in this study, it can be safely guessed that *Clo* likely contain steroidal saponins. Saponins were assessed using frothing method. Therefore, positive test observed for *Cal* could be partly due to protein/peptide present. Nevertheless, *Pgu* showed positive test for steroidal assessment. Only *Cal* exhibited effervescence on the addition of Na_2CO_3. Conclusively, it can be

Table 2. Selected physicochemical characteristics of extracts of plant foods.

Food extract	pH	[1]Acidity (x10^{-2})	[2]Relative reducing power (x10^{-4})	[3]Total phenolic (x10^{-3})
Clo	6.60±0.02^{a}	0.07±0.01^{b}	4.17±0.76b^{c}	9.95±0.56^{a}
Tco	6.40±0.01^{b}	0.10±0.00^{b}	2.84±0.57^{c}	6.00±0.00^{b}
Cal	3.00±0.00^{d}	2.43±0.02^{a}	9.46±0.76^{a}	11.67±0.56^{a}
Pgu	6.20±0.01^{c}	0.12±0.02^{b}	5.87±0.19^{b}	7.23±0.50^{b}

Values not followed by the same latter in a column are significantly (p =0.05) different. [1]g lactic acid equivalent/ml of extract; [2-3]g ascorbic acid equivalent/ml.

inferred regarding pro-fermentation capability of the extracts that, the plants food extracts could support lactic acid fermentation such as found in yoghurt in the following order: *Clo* > *Cal* > *Pgu* > *Tco*. In addition, phytochemical result shows that the tested samples are endowed with some bioactive components that could exhibit antioxidants activities. This is because natural antioxidants can be phenolic compounds (tocopherols, flavonoids and phenolic acids), nitrogen compounds (alkaloids, chlorophylls, amino acids, amines, peptides) or carotenoids as well as ascorbic acids (Velioglu et al*.,* 1998). Bringing to mind, the biological functions of antioxidant are notably antimutagenicity, anticarcinogenicity and anti-aging (Cook and Samman, 1996). The bioactive component of the extracts (*Clo*, *Cal*, *Pgu* and *Tco*) may contribute to the maintenance of good health when ingested.

Selected physicochemical characteristics of extracts of the plant foods

Selected phytochemical characteristics of the plant food extracted are presented in Table 2. This is important to gain insight to pro-fermentation potentials of the bioactive components of the plants food extracts for yoghurt preparations. The pH of *Cal* suggests that the extract is compatible with fermentation condition required for preparation of yoghurt. This is because an acidic (low pH) or reduction support-environment is necessary for multiplication of the fermentation organisms (Potter and Hotchkiss, 1996; Ihekoronye and Ngoddy, 1985). However, none of the extracts (Table 2) was characterized with alkaline pH. This suggests that none of the extracts was expected to inhibit fermentation of yoghurt on the basis of pH. Titratable acidity of the extracts correlates well with the pH. The test positive of Na_2CO_3 on *Cal* concur with (least) pH in acid region as well as its highest titratable acidity.

Relative reducing power of the extracts correlates positively (r = 0.91) with titratable acidity of the extracts. Therefore, it can be speculated that reduction is accomplished by proton abstraction from bioactive components of food extracts. Total phenolic content (x 10^{-3} g ascorbic acid equivalent/ml) was found to be highest (11.67) for *Cal* and least (6.00) for *Tco.* However, all the samples possessed reasonable amounts of phenolics. Positive correlation (r = 0.75) was found for total phenol content and relative reducing power.

Phenolic contents and relative reducing power are two fundamental antioxidative markers in plant foods (Amarowicz et al*.,* 2000). Therefore, their evaluation could be used as indication of antioxidant potentials of the plants foods extracts. Consequently, the antioxidative markers were adopted for guessing the health promotion/maintenance potentials of the food extracts used in this study.

All the extracts absorbed (nm) well (332, 342, 336 and 369 for *Tco, Cal*, *Pgu* and *Clo*, respectively) at UV region. Strong absorption in the UV region by extracts is a diagnostic feature of non-bonded electron or un-saturation in the absorbing molecule (Shriner et al*.,* 1979) and free-election is a pre-requisite for anti oxidant activity (Giese, 1996).

Physicochemical characteristics of plant food extract modified and plain yoghurt

The results of the physicochemical characteristics of plant food extracts modified yoghurt and plain yoghurt are shown in Table 3. In corporation of plant food extract into milk prior to fermentation to yoghurt gave high (1.08 to 1.30) titratable acidity (g lactic acid equivalent/ml) of modified yoghurt in comparison to low (1.02) titratable acidity of plain yoghurt. Increase in titratable acidity is thought to be due to enhanced redox potentials of the substrates influenced by bioactive components in the plants food extracts. *Cal* - YOG possessed the highest amount of titrated acidity. This result is not unexpected because the food extract is associated with the highest amount (1.30) of titratable acidity (Table 2) among the plant food extracts used on this study. The values of pH (Table 3) of *Clo* – YOG and *Cal*-YOG were within stipulated range for yoghurt preparation (Posecion et al*.,* 2005). However, the pH value of *Tco* – YOG, though lower, but sensorial assessment (Table 4) revealed that the product was not favoured by panelist, in all the parameters evaluated. This suggests that the nature of fermentation that occurred was not desirable for yoghurt preparation. Comparing this result with the phytochemical (Table 1) endowment of the extracts, it appears that

Table 3. Physicochemical characteristics of plant food extracts modified and plain yoghurt.

Sample	[1]Titratable acidity ($x10^{-2}$)	pH	Water holding capacity (%)	[2]Total phenolic content ($x10^{-3}$)	[3]Relative reducing power ($x10^{-3}$)	Relative viscosity (ηrel)
YOG	1.02±0.09^{b}	4.50±0.01^{ab}	77.44±6.00^{a}	45.00±0.05^{b}	1.38±0.03^{b}	1.17±0.02^{a}
Tco-YOG	1.20±0.02^{a}	3.80±0.20^{b}	78.40±4.80^{a}	16.3±0.00^{c}	0.7±0.02^{c}	1.08±0.01^{b}
Clo-YOG	1.08±0.00^{b}	4.80±0.20^{a}	88.51±480^{a}	140±0.15^{a}	9.53±0.50^{a}	1.23±0.00^{a}
Cal- YOG	1.30±0.20^{a}	4.30±0.20^{ab}	85.99±510^{a}	50.00±0.05^{b}	9.56±0.05^{a}	1.08±0.00^{b}
Pgu- YOG	1.15±0.01^{a}	5.00±0.10^{a}	85.44±4.30^{a}	48.00±0.5^{b}	1.53±0.05^{b}	1.20±0.00^{a}

YOG, Plain yoghurt; *Tco*- YOG, *T. conophorum* extract modified yoghurt; *Clo*- YOG, *C. longa* extract modified yoghurt; *Cal*- YOG, *C. albidum* extract modified yoghurt; *Pgu*- YOG, *P. guineese* extract modified yoghurt. [1]Lactic acid equivalent/ml sample; [2,3]ascorbic acid equivalent/ml. Any two means in a column not followed by same letter are significantly (p=0.05) different.

Table 4. Sensorial scores of food extracts modified and plain yoghurt.

Quality parameter	Sample				
	***Tco*-YOG**	***Clo*-YOG**	***Cal*-YOG**	***Pgu*-YOG**	**YOG**
Chroma	2.44^{d}	8.55^{a}	4.33^{c}	1.89^{d}	6.75^{b}
Odour	4.11^{abcd}	5.33^{abc}	6.33^{a}	1.33^{d}	5.89^{ab}
Colour	1.33^{c}	7.44^{a}	2.89^{c}	2.78^{bc}	8.55^{a}
Creaming property	1.11^{b}	7.44^{a}	3.22^{b}	3.33^{b}	7.89^{a}
Taste	2.00^{c}	7.50^{a}	7.30^{a}	6.45^{b}	7.45^{a}

Lower value implies low preference for quality. Any two means in a row not followed by same letter is significantly (P=0.05) different.

alkaloids may not possess pro-fermentation attribute for yoghurt.

Water holding capacity (WHC; %) result revealed that *Clo*-YOG, *Cal*-YOG, and *Pgu*-YOG had the highest amounts (in decreasing order) of WHC comparatively to YOG and *Tco*-YOG. High WHC of the preparations compared to YOG signified that the bioactive components of the plant food extracts facilitated cross-linkage of water molecules and food molecules (starch, protein and their oligomers). The reason for this assertion can be expressed on the basis of the fact that phenolics, notably flavonoids are known to possess hydroxyl moiety that can form linkage with water molecules using hydrogen bonds. For example, ferulic acid, a flavonoid was reported by Ou et al. (2001) to enhance WHC of starch. In addition, other moieties that can absorb water are present in sugar and peptide molecules. These compounds (sugars and peptides) possessing hydratable moieties were detected in high degree in *Clo* and *Cal.* Interestingly, the compounds (sugars and peptides) containing the hydratable/hydrophilic moieties were not adequately detected in *Tco* and this consequently account for the low WHC of *Tco* – YOG. The result obtained in this study is similar to earlier study by Mocanu et al. (2010) that fermentation profile of milk can be altered when such milk is supplemented by plant food extract prior to fermentation. In their study, the authors used biberry (*Vaccinium myrthillus*) and liquorice (*Glycyrrhiza glabra*) extracts, both plant extracts enhanced fermentation process as shown by increase in lactic acid and reduction in pH of the extract added samples in comparison to the sample without plant extract. Also, improvement in water holding capacity was observed. The total phenolic contents of all the plant food extracts

modified yoghurt were higher than the total phenolics content of YOG. The total phenolic content of *Tco*-YOG was exceptional to this assertion. The extreme low total phenolic content of *Tco*-YOG when compared to YOG is an indication that the biochemical conversion during fermentation was poor or hindered; therefore, there was no desirable fermentation. The values (1.53 to 9.56) of relative reducing power of plant food extract modified yoghurt were higher than the value (1.38) of YOG. However, an exception of this result was the value (0.7) of relative reducing power of *Tco*-YOG. A comparative analysis of the result of total phenolic content and relative reducing power showed that relative reducing power of the bioactive components in *Cal*-YOG was highest. High relative reducing power in *Cal*-YOG could be due to synergistic interaction between phenolics and non-phenolic reductones. Wanasundara et al. (1994) stated that synergism of phenolics with one another or and other components promotes antioxidants activity.

Relative viscosity results show that *Cal*-YOG and *Pgu*-YOG were characterized by higher relative viscosity relative to viscosity of YOG. The low relative viscosity of *Cal*-YOG in comparison to relative viscosity of *Clo*-YOG and *Pgu*-YOG could be due to the high titratable acidity or low pH of the plant food extracts. This is because Belitz and Grosch (1999) observed that acidity disrupt glycosidic linkages, limiting swelling and lowering final viscosity of starch products.

Sensorial evaluation

The result of the evaluation of effect of the plant food extracts on sensorial attributes of products (modified yoghurt) of the preparations with reference to plain (unmodified) yoghurt (YOG) is presented in Table 4. Descriptively, the hue of the yoghurt preparations modified using the food extracts were bright butter colour for *Clo*-YOG, cream colour for *Cal*-YOG, milk natural colour for *Tco*-YOG, chocolate colour for *Pgu*-YOG and off white colour for the reference (YOG). This description is important in that application of the plant food extracts resulted to a spectrum of hue in the preparations.

The colour in its descriptive terms (hue/value/chroma) of *Clo*-YOG compares significantly better than all the other samples (reference sample (YOG) inclusive). Similarly, *Clo*-YOG and *Cal*-YOG and *Pgu*-YOG were least with the reference (YOG) in terms of taste while *Tco*-YOG and *Pgu*-YOG were least preferred. Regarding creaming property, only *Clo*-YOG compared favourably with YOG among all the samples. Considering the weight of the score, it appears that *Clo* has the tendency to improve creaming property of yoghurt. The creaming property enhancement could be explained in terms of inclusion complex by bioactive components in *Clo* because the active components (phenolics) in *Clo* are capable of forming clathrate compounds within the helix of small molecules such as starches, peptides/proteins, consequently influencing creaming characteristics. The odours of the samples were not intense; the score revealed that *Tco*-YOG and *Pgu*-YOG were least preferred. The colour of YOG and *Clo*-YOG were preferred to the other samples.

Considering the stated results, *Clo* and *Cal* modified yoghurt compared favourably with the control sample (yoghurt without plant food extract) and sometimes better with respect to sensorial attributes. Therefore, the plant food extracts can be used to modify milk substrate for preparation of yoghurt without adversely affecting the aesthetic appeal of the product. However, *Tco* and *Pgu* gave no satisfactory result under the conditions employed in this study.

Conclusions

Application of extracts of plant foods such as *C. longa, and C. albidum* endowed with pro-fermentation phytochemicals notably flavonoids, reductones, peptides and metabolisable sugar could be used for the production of yoghurt with improved physicochemical, sensory and healthier food image. This preliminary study should stimulate interest to comprehensive study on the use of extracts of food of natural origin for preparation and enhancement of yoghurt quality.

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