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Physicochemical, Microbiological and Sensory Properties of Pineapple (*Ananascomosus* (L.) Merr.) Flavoured Yoghurt

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Abstract – **The aim of the present study was to evaluate the physicochemical, microbiological and sensory properties of pineapple flavoured yoghurt. Four yoghurt treatments were prepared by incorporating pineapple puree at different levels: 0% (T0 as control), 5% (T1), 10% (T2) and 15% (T3). All the yoghurts samples were analysed for their physicochemical composition (Dry matter, pH, fat, titratable acidity, solid non-fat, ash, sugar and viscosity), microbial (total bacteria count, total coliform count, yeasts and moulds count) and sensory properties (odour, colour, texture and taste). Titratable acidity and pH were also evaluated within 15 days of storage. The results of the present study showed that pineapple puree affected the physicochemical properties of yoghurt by a reductionof pH, fat, ash and viscosity and by increasing the titratable acidity, solid non-fat and sugar content. Reduction of pH and increase in titratable acidity was noted for all the yoghurt treatments during storage. The Total Bacteria Countof the yoghurt samples were noted to decrease in the presence of pineapple puree; addition of 15% of pineapple puree induced a reduction of yeasts and moulds count whereas 5% and 10% rather caused an increasein yeasts and moulds. The odour, colour and texture of all the yoghurt treatments were similar when analysed sensorially, meanwhile, in term of taste, the treatment T1 (yoghurt with 5% of pineapple puree) was more appreciated. In the nutshell, pineapple flavoured yoghurt should be encouraged for consumption.**

Keywords – **Flavoured Yoghurt, Pineapple Puree, Physicochemical, Microbiological and Sensory Properties.**

I. INTRODUCTION

Yoghurt is a coagulated milk product obtained from lactic acid fermentation by the action of a starter culture containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. It is among the most common dairy products consumed around the world, and its sensory attributes have a large effect on consumer acceptability [1]. Yoghurt is nutritious and has unique characters attributed to the symbiotic fermentation involved in its manufacturing [2].Moreover, the presence of live active bacteria in yoghurt contributes to the nutritional and therapeutic properties.

Yoghurt is a rich source of protein, carbohydrates, vitamins, fat, phosphorus and calcium. During fermentation, there is a partial hydrolysis of protein, fat and lactose rendering the yoghurt easily digested compared to milk and recommended for those suffering from lactose intolerance. In addition to the enhancement of lactose digestion by mal digesters, the health properties of yoghurt include improvement of gastrointestinal function, decreased risk of cancer, lowering of blood cholesterol levels, improved immune response and helping the body assimilate protein, calcium and iron [3]- [5].

The production and consumption of yoghurt is growing continuously due to its therapeutic properties beside its high nutritive value [6].They are many types of yoghurt present in the market now a days but they differ from each other with respect to their chemical composition, method of production, type of flavouring agents or additives used, and the nature of the post incubation process. Flavouring is common in yoghurt manufacture in order to present products that meet the taste of consumers of different cultural backgrounds.A variety of different flavouring ingredients (fruits, natural or synthetic) are currently added to yoghurt. Fruits and vegetables are nutrient suppliers which have effects on the wellbeing of the body. The addition of fruits is said to improve the quality of yoghurt [1].

Pineapple (*Ananascomosus* (L.) Merr.) is the third most important fruit crop in the tropical and subtropical regions of the world, preceded by banana and citrus [8]. It is one of the most important commercial fruit crops in the world and is known as the queen of fruits due to its excellent flavour and taste [9].Pineapple is present in Cameroon all year round with an increase of the area of cultivation and production which moved from 3121km^2 and 44186t, respectively, in 2001 to 3509km^2 and 49925t in 2005 [10]. Due to its high water content, the fruit is highly perishable with considerable losses during harvesting seasons.

Pineapple is an important source of sugars, fibres, organic acids, some essential minerals and vitamins for human nutrition with some medicinal values[11]-[15].One healthy ripe pineapple fruit can supply about 16.2% of daily requirement for vitamin C [11]. In addition to its organoleptic properties and nutritional value, it is endowed with therapeutic, antioxidant and medicinal properties due to the presence of vitamin C (ascorbic acid), flavonoids, phenolics, organic acids such as malic acid[13], [16]-[18]. Pineapple also contains an enzyme called bromelain, a proteolytic enzyme which digests food by breaking down proteins into amino acids [18] there by being beneficial to health.

Hence, the aim of the present study is to evaluate the physicochemical, microbiological and sensory properties of pineapple flavoured yoghurt.

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II. Material and Methods

A)Preparation of Pineapple Puree

The ripe pineapple (*Ananascomosus* (L.) Merr.) fruit from Njombe (Moungo Sub-Division, Littoral Region, Cameroon) were purchased from the Bamenda Food Market in the North-West Region, and brought to the Food Technology Laboratory of the Regional Centre of IRAD - Bambui, Cameroon. The fruit was washed with tap water, peeled and sliced into small sizes getting rid of the core of about 10-15mm. It was further blended in a heavy duty blender (Waring commercial, United Kingdom). Sugar (5% (w/w)) was added to the puree obtained (blended fruit) to enhance sweetness and preservation and after mixing very well, the mixture was pasteurised by heating at90 – 95°C for 5 minutes, cooled rapidly and stored in the refrigerator (4 – 6°C) until used.

The physicochemical parameters of the pineapple puree are given in table 1 here below.

Table 1. Some physicochemical composition of pineapple puree

pH	Dry matter (%)	Sugar °Brix)	Ash (%)
3.36±0.11	21.50±0.18	21.16±0.28	0.20±0.03

B) Preparation of Pineapple Flavoured Yoghurt

Fresh cow's milk collected from the dairy unit of the Regional centre of IRAD - Bambui was used to produce the yoghurt samples. The yoghurt production technology was as in [19] with some modifications. The milk was pasteurised by heating at 85-90°C for 5min in a boiling water bath during which sugar was added at the rate of 6.5% (w/v). The milk was then rapidly cooled to inoculation temperature (42 – 45°C) followed by addition of 2.5% (v/v) yoghurt starter culture (CHR HANSEN YF – L811) comprised of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in the ratio of 1:1. The pineapple puree was then added and incubation at42- 45°C for 3 hours for the yoghurt to set. Four yoghurt treatments were obtained as follows: T0; T1; T2 and T3 with 0, 5, 10, and 15% of pineapple puree (w/v), respectively. These samples (treatments) were subjected to physicochemical, microbiological and sensory analyses.

C) Physicochemical Analysis

The physicochemical analysis of each sample were done in duplicate.

The dry matter (DM), pH, titratable acidity (TA), ash and fat were determined according to the standard AOAC methods [20]. pH and titratable acidity determination were also done within 15 days of storage under refrigeration conditions (4 – 6°C) at 5-day interval.

The sugar content was measured at 20°C with an eclipse refractometer (Belligham + Stanley Ltd, United Kingdom) and the result expressed in °Brix (%sucrose). The viscosity was determined with a viscometer and expressed in seconds(s) needed for ball to drop through yoghurt to the bottom of the viscometer. The Solid Non Fat (SNF) was determined from the fat content according to the formula: SNF(%) = dry matter (%) – fat content (%).

D) Microbiological Analysis

Ten grams of each sample were weighed aseptically and added to 90ml of sterile diluent (0.1% peptone solution) and mixed well to give a10^{-1} dilution. By using sterile pipette, 1ml was transferred to a test tube containing 9ml of sterile diluents and mixed well to give 10^{-2} dilution. Subsequent serial dilutions up to 10^{-7} were made.

Total Viable Count was determined using the pour plate technique. The plates were incubated at 35 - 37ºCfor 24 - 48h and colony forming units per milliliter (cfu/ml) of sample estimated. For Yeasts and Moulds count, the above procedure was repeated using Potato Dextrose Agar and incubation was done at 25ºC for 72 - 120h, while for total coliforms Mac Conkey Agar was used and incubation done at 35 - 37ºC for 24 - 48h.

E) Sensory Evaluation

The yoghurt samples were held at 4 – 6±2°C until presented for sensory evaluation. A 12-member panel who were regular yoghurt consumers consisted of researchers and technicians of the centre of IRAD – Bambui. Evaluation was done on a Nine-Point hedonic scale. The scale and categories are as follows: Excellent=9; Very Good=7; Good=5; Fair=3 and Poor=1. Characteristics evaluated included odour, colour, taste and texture.

F) Data Analysis

The Data obtained were expressed as Mean±SD. One-way analysis of variance (ANOVA) and test of Least Significant Difference ($p \leq 0.05$) were used to analyse the results from all the tests using Statgraphics Plus, Version 5.0 statistical package.

III. Results and Discussion

A) Physicochemical Composition of Pineapple Flavoured Yoghurt

The physicochemical composition of the yoghurt treatments is presented in table 2 below. The control sample revealed a high ($p \leq 0.05$) pH value (4.46±0.05) and this value was reducing with increase in pineapple puree in the yoghurt. The pH values of all the yoghurt samples were higher than 4.1, the normal pH value of yoghurt but lower than 4.6 as in [19], [21]. The decreased value observed with an increase of pineapple fruit puree was expected due to the fact that the fruit puree is a sugar source and this could increase the activity of lactic acid bacteria. Moreover, pineapple is rich in organic acid and the fruit puree had a low pH (3.36±0.11); this could also contribute to the pH reduction. This result is consistent with the ones of previous studies in [7], [22].

Titratable acidity of the control sample (0.81±0.01%) was the lowest ($p \leq 0.05$) compare to other samples while sample T3 had the highest ($p \leq 0.05$) value (0.96±0.03%). The values of titratable acidity from the present study is closer to the normal value which ranges between 0.9 to 1% and the value proposed by the FDA (≥ 0.9%) [21]. Addition of pineapple fruit puree is observed to increase the titratable acidity of yoghurt. This result is similar with that obtained by previous findings in [7]. References [7], [23] indicated that fruits positively affect the titratable acidity of yoghurt. In fact, they are rich in fermentable

sugars that increase the activity of lactic acid bacteria and subsequently lactic acid production; also they contain organic acid. Reference [24] indicated a decrease of titratable acidity in fruit flavoured yoghurt compared to plain and this can be due to the type of fruit or/and the fruit preparation.

Among all the experimental samples, only sample T3 presented a different ($p \leq 0.05$) value of dry matter (18.86±0.28%) compared to the control one (18.00±0.41%). The comparable values of dry matter observed between the treatment T0 and treatments T1 and T2 may be related to the dry matter of the fruit preparation (21.50±0.18%) which was not too high compared to the one of the control yoghurt (18.00±0.41%). Reference [24] obtained a similar result with yoghurt samples collected from retail outlets in Sabaragamuwa Province of Sri Lanka whereas in [7], [22], the result was different. This differences can be explained by the type of fruit or the fruit preparation.

The fat content of the control treatment was ($p \leq 0.05$) lower than the ones of treatments T2 and T3 but comparable ($p > 0.05$) with the value of treatment T1. There was a decrease of fat content when the pineapple puree concentration in yoghurt was increasing as in [7], [24]. This is due to the fact that fruits are also characterized by their low fat content.

SNF value of treatment T3 (16.03±0.29%) was the highest ($p \leq 0.05$) and all the experimental treatments presented a high value ($p \leq 0.05$) compared to the control (14.37±0.32%). Increase of SNF in yoghurt with addition of pineapple puree was expected because the fruit has more Solid-Non-Fat than fat. Reference [22] also obtained a consistent result.

Control yoghurt with 0.67±0.00%as ash content presented the highest ($p \leq 0.05$) value and yoghurt T3 with 0.60±0.02%, the lowest ($p \leq 0.05$) value. The results indicated a reduction of ash content with an increase of pineapple puree in yoghurt. This could be explained by the low ash content (0.20±0.03%) in pineapple puree compared to the control yoghurt. Reference [23] indicated a similar result while in [7], [24] the ash content was increased in fruit flavoured yoghurt.

The sugar content of the control yoghurt was comparable ($p > 0.05$) with the one of yoghurt T1 but lower ($p \leq 0.05$) than the ones of T2 and T3 which were similar ($p > 0.05$). The present result was expected considering the sugar content of the pineapple fruit puree (21.16±0.28°Brix) and the fact that pineapple is rich in sugar content.

Addition of fruit puree reduces the viscosity of yoghurt. This value decreased ($p \leq 0.05$) with respect to increase in the fruit puree in yoghurt. This result could be related to the dry matter content of the samples. In fact, the viscosity of yoghurt increases with yoghurt dry matter. The increase of the percentage pineapple fruit was noted to correlate with that of the dry matter although differences were observed only between treatments T0 and T3.

Table 2. Physicochemical composition of pineapple flavoured yoghurt

	Yoghurt Treatments			
Parameters	T0	T1	T2	T3
pH	4.46±0.05a	4.38±0.02ab	4.33±0.05bc	4.25±0.05c
Titratable acidity (%)	0.81±0.01c	0.86±0.01b	0.89±0.01b	0.96±0.03a
Dry matter (%)	18.00±0.41b	18.39±0.41ab	18.56±0.44ab	18.86±0.28a
Fat (%)	3.63±0.15a	3.36±0.20ab	3.10±0.26bc	2.83±0.15c
SNF (%)	14.37±0.32c	15.03±0.24b	15.46±0.24b	16.03±0.29a
Ash (%)	0.67±0.00a	0.65±0.01ab	0.63±0.00b	0.60±0.02c
Sugar (°Brix)	12.66±0.38c	13.16±0.28bc	13.75±0.25ab	14.16±0.52a
Viscosity (s)	4.11±0.09a	3.74±0.21b	2.96±0.13c	2.00±0.09d

(a,b,c): The values with the same letters in the same column are not significantly different ($p > 0.05$)

B) pH Changes of Pineapple Flavoured Yoghurt During Storage

Table 3 below presents the results obtained from pH analysis of yoghurt within 15 days of storage. The results indicated a reduction ($p \leq 0.05$) of pH between the initial value (day 0) and the final value (day 15) across all the yoghurt samples. The pH is seen to decrease during storage. However, the change observed were not different ($p > 0.05$) from day 5 to day 10. This result is consistent with studies in [7], [22] but is different with the one of other studies which indicated no significant change of pH of yoghurt samples stored for 3 weeks [25]. The decrease in the pH of yoghurt, throughout its shelf life, may be due to the activity of lactic acid bacteria which is low but not stopped during storage at refrigerated conditions. Moreover, the pH value of yoghurt also depends of the availability of nutrients to lactic acid bacteria and the nutrient composition.

Table 3. pH changes of pineapple flavoured yoghurt during 15 days of storage

Yoghurt Treatments	DAY 0	DAY 5	DAY 10	DAY 15
T0	4.46±0.05a	4.35±0.05b	4.3±0.05b	4.18±0.02c
T1	4.38±0.02a	4.25±0.05b	4.23±0.11b	4.11±0.02c
T2	4.33±0.05a	4.21±0.02b	4.18±0.02b	4.1±0.05c
T3	4.25±0.05a	4.16±0.02ab	4.15±0.05b	4.03±0.05c

(a,b,c): The values with the same letters in the same row are not significantly different ($p > 0.05$)

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C) Titratable Acidity Changes of Pineapple Flavoured Yoghurt During Storage

Table 4. Lactic acid content (%) changes of pineapple flavoured yoghurt during 15 days of storage

Yoghurt Treatments	DAY 0	DAY 5	DAY 10	DAY 15
T0	0.81±0.01c	0.88±0.04b	0.96±0.02a	1.00±0.04a
T1	0.86±0.01b	0.95±0.07ab	1.01±0.08a	1.06±0.10a
T2	0.89±0.01b	0.96±0.01ab	1.04±0.08a	1.07±0.11a
T3	0.96±0.03c	1.04±0.05b	1.12±0.04a	1.18±0.03a

(a,b,c): The values with the same letters in the same row are not significantly different (p>0.05)

The change in lactic acid content of yoghurt samples during 15 days of storage is presented in the above table. There is an increase ($p \leq 0.05$) of the titratable acidity between day 0 and day 15 across all the samples. From day 0 to day10, the titratable acidity of samples T0 and T3 were increasing ($p \leq 0.05$) gradually. From day 10 to day 15, the values obtained are similar($p>0.05$) for all the yoghurt treatments. The increase of lactic acid could be related to the activity of micro organisms which continue to produce lactic acid through fermentation during storage at refrigerated conditions. In fact, during post-acidification period, the activity of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* was not completely stopped due to the availability of nutrients in yoghurt.

D) Microbiological Properties

Table 5. Microbiological properties of pineapple flavoured yoghurt

	Yoghurts treatments			
	T0	T1	T2	T3
Total Bacteria Count ($x10^{-6}$cfu/ml)	3.86±0.86a	1.66±0.24b	1.53±0.05b	1.50±0.07b
Total Coliforms($x10^{-2}$cfu/ml)	<1	<1	<1	<1
Yeasts and Moulds($x10^{-3}$cfu/ml)	27.33±4.04c	57.66±3.51a	39.33±3.05b	12.00±1.73d

(a,b,c,d): The values with the same letters in the same row are not significantly different (p>0.05)

As can be seen in table 5 above, control yoghurt (T0) significantly ($p \leq 0.05$) presented the high value of total plate count while samples T1, T2 and T3 have shown the similar ($p>0.05$) values. Addition of pineapple puree in yoghurt reduced bacteria growth. This could be related to the antibacterial effect of bromelain.

For all the yoghurt treatments, the total coliforms were lower than 10^2cfu/ml, in accordance with standards due to the fact that presence of coliforms in yoghurt may be as a result of contamination or inefficient pasteurisation. Reference [24] revealed consistent findings.

Treatments T1 indicated the highest ($p \leq 0.05$) amount of yeasts and moulds while T3 was the lowest($p \leq 0.05$).Increasing the quantity of pineapple puree in yoghurtled to a reduction of yeasts and moulds counts. The reduction observed can be attributed to the presence of bromelain also characterized by it antifungal activity. Flavouring of yoghurt with fruits enhance the growth of yeasts and moulds [23]. Nevertheless, increasing the quantity of fruits could have a negative response especially if the fruit has an antifungal component. Reference [26] indicated similar result by incorporating carrot juice in yoghurt and explained by the possible action of isocoumarine naturally present in traces in carrots.

E) Sensory Evaluation

The sensory scores obtained for all the yoghurt treatments are provided in table 6. There is no difference ($p>0.05$) among all the sample in terms of odour, colour and texture. The Control yoghurt sample showed the lowest score (5.16±2.16) about the taste attribute. This value was comparable ($p>0.05$) to the ones of yoghurt T2 and yoghurt T3 (respectively, 6.33±2.14 and 6.66±1.87) but not similar ($p \leq 0.05$) to the one of yoghurt T1 (7.00±2.08). This result is not consistent with the one obtained from previous studies which indicated a significant effect of pineapple on the yoghurt odour and flavour but no significant effect concerning the appearance, colour and texture of yoghurt [25]. The difference may be due to many factors including the treatment and the ripening stage of the fruit.

Table 6. Sensory evaluation scores of yoghurt treatments

	Yoghurt treatments			
Sensory attributes	T0	T1	T2	T3
Colour	7.33±1.88a	7.16±1.99a	7.83±1.58a	8.00±1.34a
Odour	5.83±2.16a	6.5±1.9a	6.33±1.77a	7.00±1.99a
Texture	6.83±2.32a	5.66±1.77a	5.83±1.80a	5.83±1.33a
Taste	5.16±2.16b	7.00±2.08a	6.33±2.14ab	6.66±1.87ab

(a,b): The values with the same letters in the same row are not significantly different ($p>0.05$)

IV. Conclusion

In this study, pineapple puree affects the physicochemical properties of yoghurt by reducing the pH, fat content, ash content and viscosity on the one hand and on the other hand by increasing the titratable acidity, solid non-fat and sugar content especially at 10% and 15%. Addition of pineapple puree in yoghurt reduces the total bacterial count and at 15%, also reduces the development of yeasts and moulds. Sensory properties of yoghurt remained unchanged by flavouring with pineapple puree except the taste which has been enhanced with 5% of pineapple puree.

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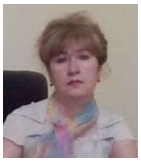

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