BEST INCLUSION RATE OF PURPLE YAM "*Kinampay*" (*Dioscorea alata* L.) POWDER IN YOGHURT

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ABSTRACT

The study was conducted to develop a technology for the supplementation of yoghurt with purple yam powder (PYP) as a potential source of prebiotic and determine its effect on the properties of the product. Five inclusion rates of gelatinized PYP were used: 0% (Control), 2% (T_1), 3% (T_2), 4% (T_3) and 5% (T_4). Higher acidity level was observed in T_1, T_2, T_3, and T_4 compared to control throughout 1, 7 and 14 days of storage. No significant differences were observed in terms of fat, protein, moisture and total solids. Yoghurt supplemented with gelatinized PYP showed a higher viable cell count of *Lactobacillus delbrueckii* subsp. *bulgaricus* compared with the unsupplemented yoghurt. This indicates the positive influence of gelatinized PYP on the growth of the bacteria due to its prebiotic properties. Sensory characteristics of yoghurt enriched with 2% gelatinized PYP (T_1) had higher overall acceptability and sensory scores in terms of aroma, sourness, sweetness, texture, body and consistency, and flavor than the control, T_2, T_3, and T_4. The results show that the best inclusion rate of gelatinized PYP in yoghurt is 2% and supplementation with purple yam boosts the characteristics of yoghurt as functional food.

Key words: *kinampay,* prebiotic, purple yam powder, yoghurt

INTRODUCTION

The addition of prebiotic to food products have become a more common occurrence over recent years due to its potential role in human health and disease prevention. Prebiotic, a short-chain carbohydrate which enables the specific changes in the composition and activity of the gut microorganism, is a selectively fermented ingredient that confers a benefit on the host's well-being and health (Gibson *et al*., 2004). Generally, carbohydrates such as inulin and its hydrolysates, fructooligosaccharides (FOS), galactooligosaccharides (GOS), resistant starch, and lactulose are the prebiotics normally used in the manufacture of yoghurt and other fermented milk (Nobakhti *et al*., 2009). Since the discovery of prebiotics as health-promoting food ingredients, the interest of the consumers continued to increase.

Food starches are extracted from a wide variety of available and affordable dietary staples such as cereals, fruits, vegetables, legumes, roots and tubers, and are used in yoghurt

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manufacture. This is because the starches give bulk to the product and improve the texture and nutritional value. Given this statement, oligofructoses and starch appeared to be an important food ingredient that could be continuously explored in the development of functional foods (Benkeblia, 2013). Among other plants which are being considered as a potential source of prebiotics, tubers and root crops such as purple yam (*Dioscorea alata* L.) stand out as an interesting candidate for industrial productions as it could accumulate the amount of resistant starch and dietary fiber.

Purple yam, *Dioscorea alata* L., is a valuable source of carbohydrates especially in arid regions (Andres *et al*., 2017). Apart from some of the documented physiological functions of purple yam, few studies have been conducted to determine the properties of purple yam used as functional ingredients. Some studies have been investigated to determine the prebiotic effect on yam through *in vitro* and *in vivo* experiments. Hsu *et al*. (2006) analyzed the prebiotic effect of yam when it was applied *in vivo* to mice. Results showed that the intake of yam inhibited the growth of *Clostridium perfringens* while the growth of *Bifidobacterium* and *Lactobacillus* have been enhanced in the gut of mice. This only indicates that intake of yam could effectively change intestinal microflora variation.

Yoghurt, a fermented dairy product, has been used in managing intestinal disorders by improving the intestinal microflora and beneficially influencing the health of the host. Corriue and Beal (2016) reported that yoghurt is considered by nutritionists to have high nutritional value due to the significant concentration of calcium, and positive bioactive effects as well as the presence of live bacterial starters which makes it a preferred dairy product by the health-conscious individual. It also includes the addition of probiotics for the fermentation of milk and the addition of prebiotics that can stimulate the growth of health-promoting bacteria present human gut (Tamime and Robinson, 2007). The study aimed to determine the level of inclusion of gelatinized purple yam powder in yoghurt formulation and evaluated the physico-chemical properties, microbiological and sensory characteristics of enriched purple yam yoghurt.

MATERIALS AND METHODS

Fresh tubers of purple yam, "*Kinampay*" variety, were purchased from a local farm in San Pablo City, Laguna and characterized by the Bureau of Plant Industry. This variety was previously confirmed by Felismino (2019) to have a potential prebiotic activity. Purple yam was powdered using a method previously described by Akinwande *et al*. (2008) with some modifications. Yam tubers were washed and steamed to inactivate enzymes and allowed ease of peeling. The steamed tubers were then peeled, grated and dried in an oven dryer at 60°C overnight. The dried yam was cooled, milled by a micro mill and passed through 250 mesh sieves to obtain the flour. The powder was kept on a tightly closed container until analysis. Prior to yoghurt production, 10 g of purple yam powder was gelatinized by dissolving in 100 mL distilled water. The mixture was heated at 100°C and stirred continuously for 30 minutes or until the mixture was smooth or no grittiness was observed. The gelatinized purple yam powder was then cooled and added to milk.

Freeze-dried granules of Direct Vat Set (DVS) type YC-380 were initially prepared according to the manufacturer's description (50 units of YC-380/ 10L of milk) and was added after pasteurization and cooling of milk. A single lot of stirred yoghurt was prepared from UHT milk (3.5% fat; 3.4% protein, and 5.2% carbohydrates) purchased at the local market.

Sugar was added at 6% (w/v) followed by skimmilk powder. The gelatinized PYP was then added at different concentrations as shown in Table 1. At 90°C for 30 minutes, milk was pasteurized and immediately cooled to 42°C with continuous stirring. The previously weighed starter culture was added to milk. The inoculated mixture was then placed in plastic containers with a tight seal and incubated at 42°C for 6 hours until pH 4.6 (pH end point) was reached. After the desired pH was reached, yoghurt was cooled and kept at 7°C. The finished products were analyzed for physico-chemical properties, sensory, and microbiological characteristics.

The physico-chemical analyses of yoghurt samples such as pH, titratable acidity (TA), fat, protein, moisture content, and total solids (TS) were analyzed on 1, 7 and 14 days of storage following AOAC (2006).

For the sensory characteristics of yoghurt samples (control and experimental), attributes such as color, aroma, texture, sweetness, sourness, flavor, and overall acceptability were analyzed after overnight storage at 7°C. Ten experienced panelists were chosen to rate the samples using the nine-point Hedonic scale method used by Isanga and Zhang (2009). Approximately 25 mL of samples were placed on coded cups. The sensory scores were determined by giving scores based on the likeness or desirability of the panelists to yoghurt samples. Three batches were made which served as the replicates. The analysis was carried out at the Dairy Training and Research Institute, College of Agriculture and Food Science, University of the Philippines Los Baños, Laguna.

Microbial counts of *Lactobacillus delbrueckii* subsp. *bulgaricus* (*Lb. bulgaricus*) were determined using the standard pour plate technique during 1, 7, and 14 days of storage (BAM, 1998). One mL of yoghurt sample was diluted with 9 mL of sterile peptone water (0.1%) and subsequent serial decimal dilutions were employed. Microbial counts were done using the pour plate technique and MRS agar plates were used to count the *Lb. bulgaricus*. Samples were incubated at 37°C for 48 hours and typical colonies of bacteria in plates with 30-300 colonies were counted. The counts were averaged for three replicates and expressed as cfu mL^{-1}. All the experimentations were performed in triplicate and the mean values were reported.

All experimental results were performed in triplicate wherein each batch of production was considered as replicate. Data on physico-chemical and microbiological characteristics were analyzed separately for each storage time by one-way Analysis of Variance (ANOVA) in Completely Randomized Design using SAS Version 9 (SAS Institute, Cary, NC, USA). On the other hand, the data on sensory evaluation was subjected to

Table 1. Experimental treatments for the level of inclusion of gelatinized purple yam powder in yoghurt.

Yoghurt Treatment	Purple Yam Powder % (v/w)
Control	-
T_1	2
T_2	3
T_3	4
T_4	5

ANOVA in randomized complete block design (RCBD) with panelists as the blocking factor using the same software. The comparison of means was done using Scheffe's test where $P<0.05$ was considered for significant difference. Results were expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Table 2 shows the physico-chemical characteristics of yoghurt supplemented with 2, 3, 4, 5% gelatinized PYP at 1, 7 and 14 days of storage. Results showed no significant difference ($P<0.05$) on the composition in terms of the fat, protein, moisture, and TS content of yoghurt samples. This is expected since similar formulations were utilized for the production of yoghurt and the only difference is the level of the gelatinized PYP added to samples.

The findings in this study also revealed that the addition of gelatinized PYP had a significant effect ($P<0.05$) on the mean pH and TA values on all yoghurt samples. According to Routray and Mishra (2011), the development of acidity in yoghurt manufacturing plays an important role in the formation of the yoghurt structure, enhancement of the bacterial growth and flavor development. As presented in Table 2, T_3 and T_4 had the maximum decrease in pH values and were significantly lower than T_1, T_2, and Control throughout storage. The results agreed with the study conducted by Mwizerwa *et al.* (2017), who investigated the effect of different concentrations (0, 0.1, 0.5, and 1%) of cassava starch on the pH of yoghurt. Their results showed a decrease in pH when the level of cassava starch increases. The activity of starter culture can be attributed to the post-fermentation acidification of yoghurt during low-temperature storage. FDA (2009) reported that a pH value of ≤4.6 is the standard pH value for fermented milk mostly yoghurt. This can be supported by the study of Hassan and Amjad (2010) who stated that the reduction of pH can be attributed to the breakdown of lactose to lactic acid. Meanwhile, TA of yoghurt samples were also significantly different ($P<0.05$) during storage. On day 1, control had the lowest TA compared to T_1, T_2, T_3, and T_4. Additionally, an increase in acidity was observed in yoghurt samples enriched with different concentrations of gelatinized PYP until 14 days of storage. The mean values of TA increase as the prebiotic concentration increases during storage. This is in accordance with the previous research of James (2003) who reported that differences in titratable acidity values could be attributed to the activity and growth of lactic acid bacteria during fermentation. A study carried out by Ceapa *et al.* (2013) showed that the presence or addition of prebiotics can increase the action mechanism of the lactic acid bacteria (LAB) to produce lactic acid since prebiotics serve as the source of nutrients to support their growth during fermentation. As stated by Singh and Byars (2009), the acidity development in yoghurt is attributed to the activity of LAB in yoghurt during storage. Lactic acid bacteria breaks down lactose into lactic acid and resistant starch of gelatinized PYP into small molecules that can be fermented into acid.

The sensory quality scores of yoghurt enriched with gelatinized PYP stored overnight at 7°C are presented in Table 3. Significant differences ($P<0.05$) were observed among all yoghurt samples in terms of color, aroma, sweetness, sourness, texture, body and consistency, flavor, and overall acceptability. Results showed that yoghurt enriched with 2% gelatinized PYP obtained the highest score and highly acceptable product based on the scores given in terms of its sweetness, sourness, texture, body and consistency, flavor, and overall acceptability.

Table 2. Physico-chemical characteristics of yoghurt enriched with different levels of inclusion of gelatinized purple yam powder.

Treatments	Storage Time (days)		
	1	7	14
pH			
Control	4.56 ± 0.03[a]	4.35 ± 0.05[a]	4.15 ± 0.06[a]
T_1	4.48 ± 0.15[b]	4.25 ± 0.12[b]	4.00 ± 0.05[ab]
T_2	4.32 ± 0.11[b]	4.21 ± 0.09[b]	3.95 ± 0.06[b]
T_3	4.30 ± 0.10[b]	4.10 ± 0.07[c]	3.90 ± 0.15[b]
T_4	4.28 ± 0.13[b]	4.08 ± 0.16[c]	3.90 ± 0.09[b]
P-value	0.0181	0.0252	0.0322
Titratable Acidity			
Control	0.72 ± 0.28[c]	0.99 ± 0.14[c]	1.95 ± 0.21[b]
T_1	0.82 ± 0.17[b]	1.21 ± 0.07[bc]	1.96 ± 0.04[b]
T_2	0.86 ± 0.12[ab]	1.50 ± 0.10[ab]	2.10 ± 0.03[ab]
T_3	0.89 ± 0.12[a]	1.89 ± 0.09[a]	2.18 ± 0.08[a]
T_4	0.89 ± 0.13[a]	1.90 ± 0.03[a]	2.21± 0.08[a]
P-value	<0.0001	0.0121	0.0476
Fat Content (%)			
Control	3.52 ± 0.05	3.53 ± 0.08	3.51 ± 0.07
T_1	3.51 ± 0.10	3.48 ± 0.07	3.45 ± 0.04
T_2	3.51 ± 0.08	3.49 ± 0.14	3.45 ± 0.13
T_3	3.50 ± 0.13	3.45 ± 0.10	3.45 ± 0.12
T_4	3.51 ± 0.05	3.48 ± 0.06	3.48 ± 0.10
P-value	0.8897	0.7546	0.6987
Protein Content (%)			
Control	3.57 ± 0.16	3.55 ± 0.09	3.55 ± 0.13
T_1	3.54 ± 0.14	3.52 ± 0.11	3.50 ± 0.17
T_2	3.54 ± 0.05	3.52 ± 0.04	3.50 ± 0.07
T_3	3.53 ± 0.14	3.52 ± 0.21	3.49 ± 0.24
T_4	3.53 ± 0.05	3.51 ± 0.07	3.49 ± 0.11
P-value	0.6091	0.7951	0.6643

Table 2. Continued...

Treatments	Storage Time (days)		
	1	7	14
Moisture Content (%)			
Control	85.17 ± 0.10	85.10 ± 0.12	85.08 ± 0.08
T_1	85.26 ± 0.18	85.21 ± 0.23	85.18 ± 0.25
T_2	85.29 ± 0.08	85.23 ± 0.13	85.20 ± 0.19
T_3	85.35 ± 0.16	85.30 ± 0.09	85.28 ± 0.11
T_4	85.40 ± 0.18	85.35 ± 0.19	85.32 ± 0.22
P-value	0.7717	0.1325	0.0826
Total Solids Content (%)			
Control	17.47 ± 0.06	17.40 ± 0.03	17.36 ± 0.07
T_1	17.75 ± 0.08	17.70 ± 0.11	17.67 ± 0.09
T_2	17.77 ± 0.07	17.72 ± 0.06	17.69 ± 0.08
T_3	18.08 ± 0.11	17.96 ± 0.17	17.85 ± 0.15
T_4	18.11 ± 0.20	18.12 ± 0.14	17.89 ± 0.12
P-value	0.7719	0.1452	0.0765

[abc]Means with different superscripts within the same column are significantly different ($P<0.05$)

In terms of sourness, there was an increase in the acidity level with the increasing level of gelatinized PYP on yoghurt samples. This only showed that the level of sourness of yoghurt with 2% gelatinized PYP was still highly acceptable than the sourness scores of the control. This could also be correlated to the sweetness of the yoghurt that decreases during storage due to the growth and activity of the starter culture thus, cause an increase in the acidity (Fellows *et al*., 1991). In this study, yoghurt with 2% gelatinized PYP had the highest overall scores since a balance between sweetness and sourness was observed. The increasing level of the gelatinized PYP also affects the consistency of the product that changes the yoghurt's thickness; and, the texture of the yoghurt product which may be due to the grittiness or graininess felt during the sensory. These attributes seem to be important factors for consumer acceptability and promotional of the products in the market hence, supplemented products remain unchanged after production and during storage (Zare *et al*., 2011).

In the current study, a decline in the acceptability was also observed with the increasing concentrations of gelatinized PYP. This may be due to the low scores in texture, sourness, body and consistency, and flavor characteristics of the yoghurt having higher concentrations. A Spearman's rank correlation however, did not show a high correlation among sensory attributes to all yoghurt samples.

It reveals in this study the possibility to produce an acceptable yoghurt enriched with gelatinized PYP even without the addition of any stabilizers. With this, yoghurt enriched with 2% PYP is the preferred best inclusion rate.

The data on the viable cell count of bacteria in all yoghurt samples enriched with gelatinized PYP during storage are presented in Figure 1. A significant difference ($P<0.05$)

Table 3. Sensory characteristics of yoghurt enriched with gelatinized purple yam powder[1].

Parameters[2]	**Control**	**T_1**	**T_2**	**T_3**	**T_4**	***P*-value**
Color	4.83 ± 0.53^d	7.53 ± 0.11^b	6.38 ± 0.85^c	7.70 ± 0.47^b	8.67 ± 0.36^a	<0.0001
Aroma	7.57 ± 0.02	7.58 ± 0.09	7.57 ± 0.01	7.51 ± 0.04	7.51 ± 0.09	0.6591
Sourness	8.32 ± 0.31^b	9.50 ± 0.57^a	7.55 ± 0.51^c	7.44 ± 0.34^c	7.28 ± 0.61^d	<0.0001
Sweetness	4.03 ± 0.74^d	9.30 ± 0.68^a	8.37 ± 0.61^b	7.87 ± 0.57^c	7.82 ± 0.36^c	<0.0001
Texture	12.13 ± 0.24^a	12.13 ± 0.74^a	9.30 ± 0.86^b	6.93 ± 0.28^b	5.10 ± 0.68^c	<0.0001
Body and Consistency	7.95 ± 0.41^b	7.98 ± 0.65^a	6.77 ± 0.81^c	6.73 ± 0.79^c	6.17 ± 0.31^c	<0.0001
Flavor	11.20 ± 0.59^a	11.90 ± 0.77^a	8.93 ± 0.53^b	7.50 ± 0.31^c	7.50 ± 0.63^c	<0.0001
Overall Acceptability	8.47 ± 0.38^c	12.70 ± 0.63^a	9.74 ± 0.36^b	7.34 ± 0.70^d	6.94 ± 0.57^d	<0.0001

[1]Samples were on overnight storage at 7°C

[2]Scores: 0=extremely undesirable; 10=extremely desirable

[a-d]Means with different superscripts within the same row differ significantly ($P < 0.05$)

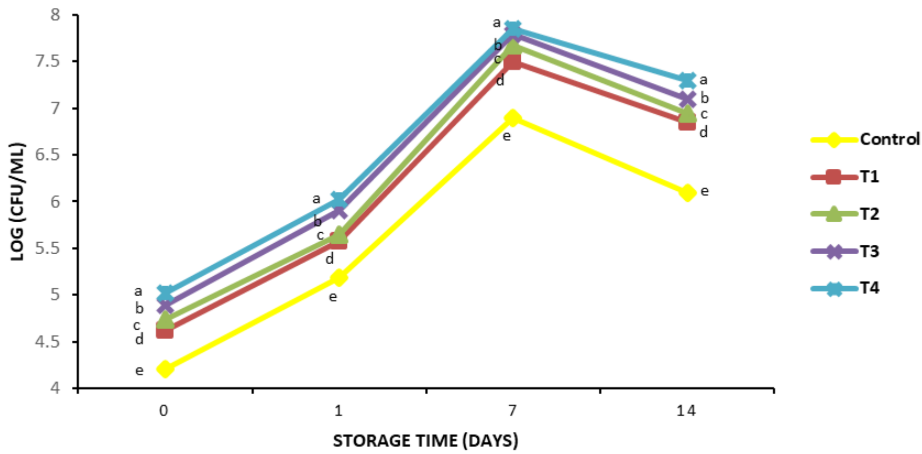

Figure 1. Effect of enriched gelatinized purple yam powder on the microbial count of yoghurt samples during storage.

on the viable count of *Lb. bulgaricus* was observed in yoghurt samples with gelatinized PYP compared to control during storage.

The results showed that the control sample exhibited significantly lower count compared to yoghurt samples supplemented with gelatinized PYP in T_1, T_2, T_3, and T_4. This only indicates that gelatinized PYP has the ability to improve the activity and stimulates the growth of the *Lb. bulgaricus*. Although all the growth factors present in milk can enhance the growth of bacteria, with the addition of prebiotics, the survival count and metabolic activity of the bacteria also increased (Akalin *et al*., 2007). This also indicates that the microorganisms present in yoghurt utilized the carbohydrates present in gelatinized PYP as their carbon source.

In this study, there was an increase in the total count of all yoghurt samples from the 1st to 7th day of storage and decreased on the 14th day of storage. The nature of the stimulatory factors of purple yam is unclear and its compounds that have a protective benefit towards the lactobacilli remain to be determined. Some studies reported that the complex carbohydrates such as raffinose, stachyose, resistant starch, sucrose, and other oligosaccharides stimulate the growth of the bacterial cultures in milk (Wang and Daun, 2004) thus, causing an increase in the count of *Lb. bulgaricus*.

Higher counts of *Lb. bulgaricus* were also observed when the level of concentration of gelatinized PYP increases although, they did not show any significant difference (P<0.05) among yoghurt samples during storage. The increase in the count of *Lb. bulgaricus* could result in the higher microbiological activity leading to the production of metabolites such as amino acids and peptides. These metabolites are important for the growth of *Streptococcus thermophilus* (*S. thermophilus*) which is also a component of yoghurt starter (Angelov *et al*., 2009). Though the effect of PYP to *S. thermophilus* was not determined in this experiment, *Lb. bulgaricus* and *S. thermophilus* which strongly coexist in milk, interact beneficially with each other. This process in which the exchange of metabolites and stimulatory factors are involved is known as proto-cooperation (Pette and Lolkema, 1950). This can be supported by the study of Delgado-Fernandez *et al*. (2019), who confirmed that prebiotic when added at high concentration in yoghurts (≥4%) significantly influenced the growth of the starter culture. The authors added lactulose as prebiotic to yoghurt that caused an increase in the cell counts of bacteria in yoghurt.

Likewise, a previous study conducted by Settachaimongkon *et al*. (2014) proved that the symbiotic relationship between strains of *Lb. bulgaricus* and *S. thermophilus* resulted in a significantly higher count or population, faster milk acidification, and production of aroma volatiles and non-volatiles metabolites which determines the fermentation process and the final product quality. Proto-cooperation is also the basis for the production of amino and organic acids and mutual metabolism which increases the growth and activity of the two species thus, giving positive effects on the fermented product. These two microorganisms change the milk compounds by their mutual growth hence, obtaining a product with a nutrition profile.

Further, a decrease in the viable count at the 14th day of storage was also observed. Even though yoghurt samples had initial bacterial counts within limits, samples enriched with gelatinized PYP still remain within the minimum level (6 log CFU/mL or higher) during 14 days of storage.

In conclusion, addition of gelatinized PYP improved the acidity, sensory characteristics and enhanced the growth of *Lb. bulgaricus* in yoghurt. The results revealed that 2% gelatinized PYP had the highest sensory acceptability scores and overall acceptability in terms of its sweetness, sourness, texture, body and consistency, flavor, and overall acceptability. This study reveals the potential of purple yam powder as a functional ingredient when added to yoghurt. Further pre-treatment analysis to purple yam powder is suggested to perform before adding to products in order to prevent its grittiness or graininess texture which could affect the sensory quality and perception of the consumers to products. It is also recommended to determine the effect of PYP on the growth of the *S. thermophilus* since it is one of the components of yoghurt starter, and to other strains of probiotic bacteria.

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