

Enhancing the Physicochemical and Antioxidant Properties of Stirred Yoghurt by Incorporating Soursop (*Annona Muricata*)

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Abstract: Stirred yoghurts incorporating with soursop juice (SJ) and soursop nectar (SN) were studied; both were added into yoghurt formulations, before and after incubation, respectively. All other ingredients and processing conditions were kept constant, and stirred yoghurt without soursop considered as the controller (CY). Result showed the physicochemical properties of two formulations complied with the Sri Lankan Standards (SLS) for fermented milk products. Highest pH (4.8) and lowest titratable acidity ($0.62 \pm 0.01\%$) were observed in SJ incorporated yoghurt (SJY) series; shown nearly two times high protein ($7.67 \pm 0.35\%$) content with compare to CY (4.34 ± 0.01). SN (15%) added yoghurt (SNY) acquired the highest sensory acceptance. Highest DPPH radical scavenging percentage was observed in SJY ($85.53 \pm 0.27\%$ to $13.87 \pm 0.93\%$) followed by SNY ($71.96 \pm 0.31\%$ to $4.13 \pm 0.00\%$) while CY had shown $17.92\% \pm 0.21\%$ to $0.44 \pm 0.04\%$ as least value. The Coliform and E-coli counts complied with the requirements of SLS though Yeast and Mold counts were slightly higher. It is concluded that SN and SJ incorporated yoghurts have shown desirable sensory properties along with enhanced physicochemical and antioxidant properties.

Keywords: Properties, Soursop, SLS, Stirred yoghurt.

I. INTRODUCTION

Yoghurt is a coagulated milk product through lactic acid fermentation of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*; vary as set, stirred, frozen, and drinking yoghurts (Tamime and Robinson, 2007). The popularity of stirred yoghurts reached to the maximum level in modern life style of world, although it has received less attention as a dairy product among the Sri Lankan. Less awareness, lack of availability and its textural characters are the major reasons. Flavored yoghurt has masked its natural sourness by used of different natural and artificial food flavors (Bylund, 2003). Incorporation of fruits as their pulps, juice, nectar, jam, jellies, etc. are the prominent among them since they have additional benefits as enhanced nutritional, functional and textural properties (Jayasinghe et al., 2015; Tarakci and Kucukoner, 2003).

The proximate composition of the yoghurt need to be contained minimum 3.25% of milk fat and 8.25% of solid nonfat (SNF) (WHO/FAO, 2011). In contrast, Sri Lankan standard (SLS) define it as minimum 3% of fat and minimum 8% of SNF (SLS 824. Part II 1989). For low fat and nonfat yoghurts, 0.5% to 2% and less than 0.5% of milk fat content was permitted, respectively. (WHO/FAO, 2011). Bovine milk or milk products are the principle constituent for traditional yoghurt production. Buffalo, goat, sheep (ovine) and camel milk also utilize in different territories (Spreer, 1998). In addition, dry milk powders, sweeteners and stabilizers are exploited. It is beneficial to present the inoculated microorganisms in the final product and need to be viable and abundant (WHO/FAO, 2011).

Soursop (*Annona muricata*) is belong to the family *Annonaceae*, and genus *Annona*; delicious fruit immigrant to Sri Lanka from the West Indies and South America in 16th century due to invaded of Portuguese. Four *Annona* spp. are commonly found in the home garden and natural forest areas in Sri Lanka; namely, *A. muricata* or soursop (Katu anoda); *A. squamosal*, sweetsop, or sugar apple (Weli anoda); *A. reticulate*, custard apple, or bullocks heart, (Matti anoda), and *A. cherimola* or cherimoya. *A. muricata* is more abundant among them and not commercially cultivated yet (Samara, 2014).

Soursop has a miraculous nature as “Natural Cancer Cell killer”, 1,000 much stronger than to chemotherapy treatment (Paull *et al.*, 2013; Preedy *et al.*, 2011), and reported to have polyketide-derived fatty acid compound called annonaceous acetogenins which can be inhibited the damaged cells just before they could become cancerous (Rupprecht *et al.*, 1990). Soursop is a juicy, acidic, and aromatic nature fruit recorded many therapeutic and nutritive properties. The fruit pulp is a rich source of fructose, and contains significant amounts of vitamins, such as C, B₁, and B₂ (Badrie and Schauss, 2010; Husni and Alias, 2009; Omoifo, 2004; Rice *et al.*, 1990). However, the well ripen fruits are highly perishable and susceptible to quick spoilage as they become easily bruised, soften rapidly may tend to mold attack easily and become spongy, and also difficult to consume as a fresh fruit (Rice *et al.*, 1990). The seasonal fruit recorded high yielding two time per year; categorized as an underutilized and neglected fruit in Sri Lanka, wasted due to spoilage or pests attacks (Dahanayake, 2015). This study was directed to determine the feasibility for utilizing soursop fruit incorporating to stirred yoghurt as SJ and SN by reducing postharvest losses of ripen fruit.

II. MATERIALS AND METHODOLOGY

2.1 Materials:

Standardized sterilized liquid milk and nonfat milk powder was obtained from local market, recorded as 3.5% and <0.05% of fat, respectively. Fully ripe soursop fruits (*A. muricata*) were obtained from the university farm. 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH), and other chemicals, from Sigma Chemical pvt Ltd (301/2, Galle Rd, Colombo-03), and all were recorded as an analytical grade. Direct-Vat-set starter (DVS) culture of *Lactobacillus* was purchased from J.L Morison Son and Jones Ceylon company, (No: 430, Colombo-15). Kjeldahl Apparatus (Digestion Unit S/N 199659, Distillation Unit S/N 205731, Water aspirator S/N 208986); Spectrophotometer (Shimadzu UV 1800) and Brookfield dual reader viscometer (Hemson international pvt Ltd, Colombo-01).

2.2 Methods:

2.2.1 Storage of Raw Materials:

Fully matured well ripen soursop fruits were collected from the university farm during the 10.00 to 11.00 hour in the morning, brought to the laboratory and subjected to 55^oC hot water treatment as surface sterilization; stored in room temperature and 7^oC in refrigerator.

2.2.2 Preparation of soursop fruit juice:

The fruit was rinsed with 45^oC slightly warm water, peeled and de-seeded by manually. Soursop extract (SE) was extracted using a mechanical blender (Kiros *et al.*, 2016); analyzed for pH, titratable acidity (TA) and, total soluble solids (TSS). Then, SE was sieved and mixed with potable water as ratio of 2:1 to formulate SJ. It is subjected to pasteurization at 85^oC for 4 minutes; sealed after hot filling to pre-sterilized 750 mL glass bottles and stored in the refrigerator.

2.2.3 Preparation of soursop nectar:

White sugar (100 g) and potable water (700 ml) were mixed together and mixture was heated to 60^oC for 10 minutes while agitating. Pre-prepared SJ (200 g) was added to the sugar solution and heated to 85^oC for 2 to 3 minutes. Sodium metabisulphate (E 223) was mixed with SN after removing from fire, hot filling was repeated and pH, TA and TSS were tested same as SJ.

2.2.4 Chemical analysis of SJ, SN and formulated Yoghurt:

pH values were measured using a calibrated digital pH meter having glass rod, calibrated with buffers of pH 4.0 and 7.0; TA was determined by titrating with 0.1N NaOH presence of phenolphthalein indicator (Richardson, 1985), and TSS content was measured as a Brix (Bx^o) value by using manual refractometer (Antago master- α , USA) as reference of AOAC, 1998 No: 932.12 method.

2.2.5 Preparation of soursop yoghurt:

All the utensils were sterilized by autoclaving. Heat liable plastic utensils were sterilized by kept in boiling water for 30 minutes (Kiros *et al.*, 2016). Nonfat milk powder (3 g, 1.35% of weight basis) was mixed with 172.46 g of pre-homogenized standardized fresh milk (77.5%); while heating the mixture, 0.68 g of gelatins (Bloom 200) (0.3%), 13 g of sugar (5.8%) and 33.38 g of pre-prepared SJ (15%) were added while continuous agitation. Then whole mixture was sterilized at 85°C for 30 minutes; allowed to cool and DVS *lactobacillus* culture was inoculated when its temperature at 44°C, incubated at 42°C for 4 hours in cooling incubator (Mammert, Compressor Cooling Incubator, ICP 260, German) and broken the coagulum and filled into pre-sterilized plastic containers. Another batch was prepared by replacing SJ by same amount of SN; added after incubation. Labelling was done at the end as 619 and 239 respectively for SJY and SNY. Controller was prepared only using milk with same constant of other ingredients. All the sample were kept at cooling incubator at 4°C.

2.2.6 Proximate composition of yoghurt:

The crude protein and ash percentage were determined by kjeldahl and Muffle furnace method (AOAC, 1998 No: 991.20 and 945.46) respectively. Moisture percentage of yoghurt was calculated by formula (AOAC, 1998 No: 990.20), (Kiros *et al.*, 2016).

$$\text{Moisture \%} = 100 - \text{Total Solid \%}$$

The crud fat content of the yoghurt was determined by using soxhlet method (AOAC, 1998 No: 905,02), (Kiros *et al.*, 2016) and solid non-fat% was calculated by deducting fat percentage from total solid content. Viscosity was measured by the method described by Igbabul *et al.*, 2014; with some modifications. No:2 spindle was used all the time, with 5, 20, and 50 rpm for 3 minutes.

2.2.7 Microbial analysis:

SJY and SNY samples were prepared based on method as; 11g of well mixed composite yoghurt sample mixed with 99 mL of peptone water at 40°C and homogenous dispersion was obtained by mixing vortex mixture (VWR 58816-120, USA) for 10 minutes (Richardson, 1985). 1:10 dilution was made up for yeast and mold count (YMC).

Qualitative determination of Coliforms was done using MacConkey agar, and pour plate technique along with red bile agar used for quantitative analysis; incubation was done 36°C for 48 hours in both experiments. Typical dark red colonies indicate the presence of coliform, and counted by using colony counter (Rocker Scientific Co, Ltd, Galaxy 230, Taiwan); (SLS 516. Part III 1982), (Richardson, 1985) and (Kiros *et al.*, 2016). YMC was determine by pour plate count method along with potato dextrose agar media (PDA), (SLS 516. Part II 1991).

2.2.8 DPPH radical scavenging activity:

15 mL aliquots were collected from the yoghurt and the pH was adjusted to 4.6 with 1 M HCl. The suspension was centrifuged at 9000 rpm⁻¹ for 20 minutes and the supernatant was filtered on a 0.45-mm filter (Gjorgievski *et al.*, 2014).

Assay was performed with fresh prepared DPPH solution (2,2 diphenyl-picryl hydrazyl hydrate) along with spectrophotometric method. 0.5 mL of aliquots was mixed with 2.5 mL of 6.5×10⁻⁵ M DPPH solution in methanol; kept in dark place for 30 minutes under room temperature and measured the absorbance at 517 nm. Experiment was organized for five concentrations and methanol was used for blank test (Perera *et al.*, 2014; Brand-Williams *et al.*, 1995). The percentage of DPPH radical scavenging activity was determined by using the equation mentioned below.

% Inhibition of DPPH absorbance A

$$A = A_C - A_S / A_C \times 100$$

A_C = Absorbance of the controller

A_S = Absorbance of the aliquots

2.2.9 Statistical Analysis:

Sensory evaluation was done with 30 untrained panel along with ballot paper prepared as five-point unipolar hedonic scale; evaluated of sensory qualities as an aroma, appearance, consistency, taste, after taste, texture and overall

acceptability. The data were analyzed by using Kruskal-Wallis non-parametric ANOVA method of Minitab 14 computer software to select the most acceptable soursop incorporate yoghurt among the SJ (code 619) and SN (code 239). Significance differences of physiochemical and proximate composition were analyzed using Duncan multiple range test in SAS 9.2 software.

III. RESULTS AND DISCUSSION

3.1 Best Storage condition:

Since soursop is a climatic fruit temperature should be well controlled after harvesting (Morton, 1987). It is recommended to store at 20°C (Pinto and Jackson, 2006). In this experiment two soursop fruits in same maturity level were stored in ambient temperature (25°C – 30°C) and refrigerated condition.

Proper maturation was observed when stored at ambient temperature; peel color was changed as dark green to pale green due to loss of chlorophyll with carotenoids. Peel was dark brown in the late ripening stage due to broken of chloroplast, and releasing polyphenol oxidases caused oxidation and polymerization of phenols (Table 1).

Based on Paull (1990), total ethanol-soluble phenol first increased (10%), and reported 50% greatest declines at after peak climacteric levels. The decline of phenols probably led to a loss of astringency taste during ripening and had reported bland flavor of the slightly overripe fruit. Over ripen fruit had an off-flavor due to low phenols, less organic acids, and fermentation of yeast and mold (Paull, 1990). Fully ripen soursop fruits stored 2–3 days only at refrigerator, afterwards seen the black color chilled injuries. The immature fruits when ripened off the tree do not develop full flavor and aroma, therefore ripening at room temperature is recommended. However, soursop pulp was reported to have 12-24 months shelf life if frozen at -18°C (Pinto and Jackson, 2006).

TABLE 1: VISUAL OBSERVATION OF SOURSOP FRUITS (IN SAME MATURITY LEVEL) AT TWO DIFFERENT STORAGE CONDITIONS

Duration	Storage in room temperature	Storage in refrigerators
After 4 days	Slightly yellow color Skin became softer. Fruit segments become wider & loosely packed.	Dark skin, failed to ripen, poor flavor and aroma, rotten easily
After 8 days	Loss of astringency and bland flavored, got brown colored skin	Off odor with rotten

3.2 Proximate analysis result:

According to the findings of Abbo *et al.* (2006), pH of soursop extract (SE) was gradually decrease with the time as 3.85 to 2.7 at ambient temperature of 28°C within first three weeks, adjoining to this initial pH. However, results were not compliance with codex standards of fruit juices and nectars as minimum brix level for SJ and minimum juice content for SN are 14.5 °Brix and 25% (v/v), respectively (table 2).

pH regulation of SJ was critical for *Lactobacillus bulgaricus* action on fermentation. *Lactobacillus bulgaricus* is homofermentative in the acidic region as 6.5 – 4.0 pH (Rhee and Pack, 1980). The pH of both SJ and SN were out of the optimum range; although pH elevated to 6.11 when it mixed with milk. Therefore, requirement of adding acid regulator was omitted.

TABLE II: SELECTED PHYSIOCHEMICAL PROPERTIES OF SE, SJ AND SN

Sample	pH	TA	TS (°Brix)	Juice percentage (v/v)
SE	3.85	1.65±0.02	14.20±0.20	100%
SJ	3.87	1.30±0.05	13.50±0.07	67%
SN	3.97	0.38±0.01	20.85±0.25	13.4%

Data represented as mean ± SE (n=3)

3.2.1 Physiochemical and proximate composition of soursop yoghurts:

pH of the SJY, SNY and CY were recorded as 4.80, 4.47 and 4.43, respectively. The results were higher than findings of Lutchmedial *et al.*, (2004); reported as pH 4.06 – 4.40 for fruit based yoghurt, and IDFA (2004) mentioned as pH 4.60 or

lower. Initial pH of milk was reduced with the addition of SJ. However, pH reduction rate during incubation was diminished. Moreover, milk casein not fully coagulate even after 6 hours incubation time (pH=4.73) (Fig. 1). The results are compatible with the findings of Mbaeyi et al. (2014), indicated that addition of soursop slightly lowered the acidity of the yoghurts. This may be due to antibacterial properties of soursop or some other microbial activity (Solís-Fuentes and del Carmen Durán-de-Bazúa, 2011) which negatively affected to lactic acid fermentation. Yet, further studies need to witness the lactic acid fermentation of soursop.

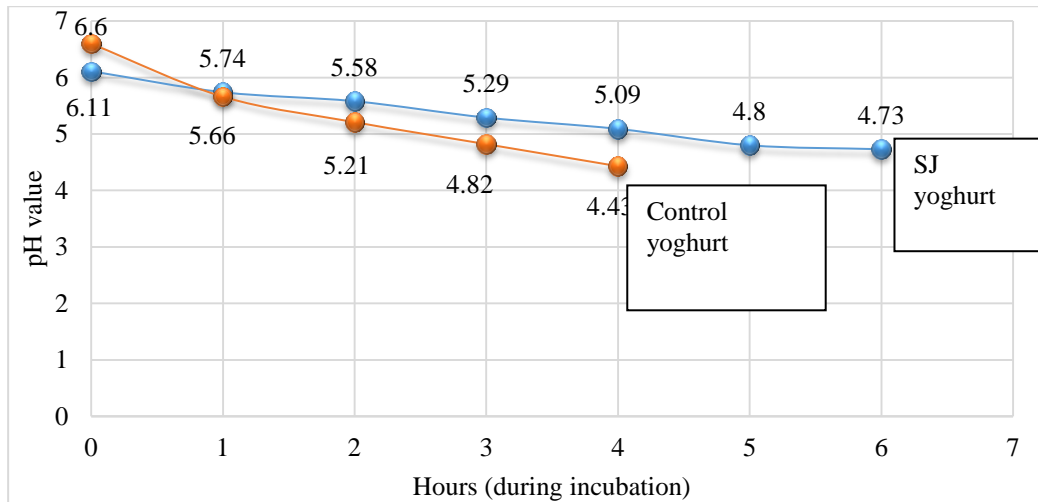


Fig. 1. pH reduction pattern of SJY and CY during incubation

TA, TSS and SNF (solid nonfat) were significantly differ among SJY, SNY and CY; compliance with SLS and codex standards (table 3). Nutritional values were complied with the SLS standards (SLS 824. Part II 1989) for yoghurt and fermented milk products in Sri Lanka. SJY was reported the highest protein content as 7.67%, although it has received low mean sum of ranks at the sensory evaluation and the moisture content was uppermost in SNY (table 4).

TABLE III: PHYSIOCHEMICAL PROPERTIES OF SJ AND SN YOGHURTS WITH COMPARE TO CONTROL

Yoghurt type	pH	TA	TSS	SNF
SLS/ Codex standards	4-4.5	Min. 0.6/0.6	-	Min. 8.00/ 8.25
Control yoghurt	4.43	0.80±0.01 ^A	20.97±0.73 ^A	16.44±0.73 ^A
SNY	4.47	0.68±0.01 ^B	18.14±0.18 ^B	13.87±0.18 ^B
SJY	4.8	0.62±0.01 ^C	19.13±0.48 ^{AB}	14.86±0.48 ^{AB}

Data represented as mean ± SE (n=3). Mean values in a column superscripted by different letters are significantly different at p<0.05.

3.3 Sensory result of SJY (Code 619) and SNY (Code 239):

Only taste and overall acceptability were significant different among the SJY and SNY (table 4). Still, Fig. 2 was pronounced that other sensory attributes also differ as the CY obtained highest mean some rank values for appearance, color, odor and texture while SNY was most preferred for taste, aftertaste and overall acceptability. Further, SJY showed the least preference for all sensory attributes. Further, SJY had not felt the unique taste and aroma of soursop. That is may be due to elevated heat treatments and fermentation. Yet, improvements should introduce to SJY production. SNY was selected as the most accepted product based on corresponding mean sum of ranks. However, it contain comparatively high amount of sugar.

TABLE IV: PROXIMATE COMPOSITION OF SJY AND SNY YOGHURTS WITH COMPARE TO CY

Yoghurt type	Protein %	Fat %	Moisture %	Ash %
SLS/ codex std.	Min. 2.7	-	-	-
Normal yoghurt (control)	4.34±0.01 ^C	4.26±0.05 ^A	79.30±0.73 ^B	0.95±0.01 ^A
SNY	6.80±0.01 ^B	4.27±0.00 ^A	81.87±0.18 ^A	0.92±0.01 ^A
SJY	7.67±0.35 ^A	4.15±0.08 ^A	80.87±0.48 ^{AB}	0.93±0.00 ^A

Data represented as mean \pm SE (n=3). Mean values in a column superscripted by different letters are significantly different at $p < 0.05$.

TABLE V: SENSORY EVALUATION RESULTS (KRUSKAL WALLIS TEST)

Attribute	Calculated H value (H_{cal})	Table H value (H_{tab})	Relationship between H_{cal} and H_{tab} values	H_0 reject/not	Significant difference exist/not
Appearance	0.31	7.82	$H_{cal} < H_{tab}$	×	×
Color	0.14	7.82	$H_{cal} < H_{tab}$	×	×
Odour	2.29	7.82	$H_{cal} < H_{tab}$	×	×
Texture	0.11	7.82	$H_{cal} < H_{tab}$	×	×
Taste	9.34	7.82	$H_{cal} > H_{tab}$	✓	✓
After taste	4.07	7.82	$H_{cal} < H_{tab}$	×	×
Overall acceptability	13.45	7.82	$H_{cal} > H_{tab}$	✓	✓

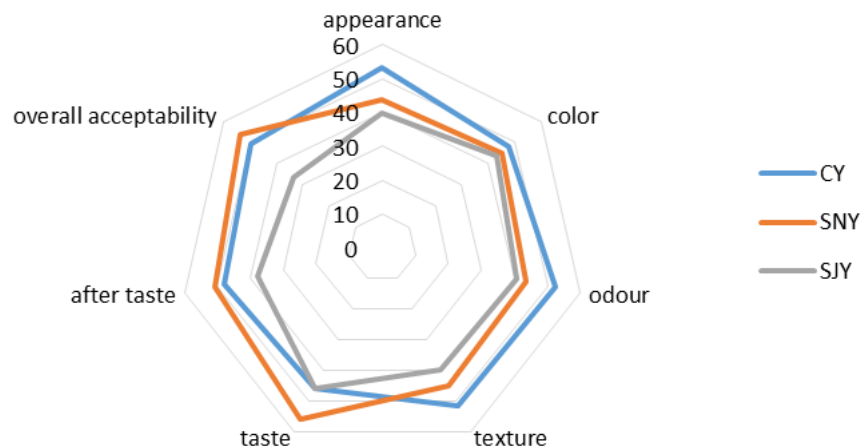


Fig. 2. Sensory profile of formulated yoghurts (based on mean sum rank values)

3.4 Microbial analysis:

YMC of SJY and SNY were exceeded the SLS standard limits. It has recommended to have less than 10 CFU g^{-1} count for yoghurt (Mostert & Jooste, 2002); although great potential to have high YMC count for fruit incorporated yoghurt (Tarakci and Kucukoner, 2003). Coliforms and E-coli were not detected in all the treatment; probably due to processed in the laboratory conditions with zero contamination.

TABLE VI: MICROBIAL ANALYSIS RESULTS FOR YOGHURTS

Yoghurt sample	Yeast count (25°C/5 days)/g		Mould count (250C/5 days)/g		E-coli, MPN/g		Coliform/g	
	I	A	I	A	I	A	I	A
SLS	<1000		<1		<1		-	
SNY	1500	1700	<100	<100	ND	ND	ND	ND
SJY	<100	<100	<10	<50	ND	ND	ND	ND
CY	12	15	5	13	ND	ND	ND	ND

ND= Not detected; I=Initial; A=after two weeks

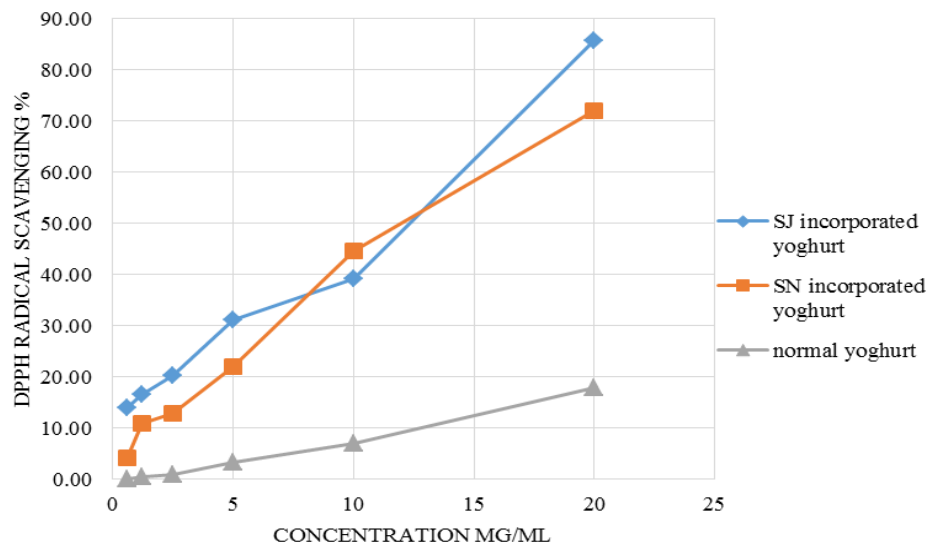


Fig. 3. Dose response relationship curves for yoghurt samples for DPPH radical scavenging activity

3.5 Free radicle scavenger power:

SJ incorporated yoghurt has reported the highest as 85.53 %, and SN yoghurt reported 71.96 %; controller reported the least radical scavenging activity as 17.92%, (Fig. 3.). It has shown high free radicle scavenger activity than finding of Gjorgievski et al., (2014) as showed as average radical scavenging activity of yoghurt was 45.18%. Results have proven soursop incorporation have positive effect on the radical scavenging activity. Further studies can be conducted to reveal the effect of temperature on free radical scavenging power of soursop to alter the thermal treatments during processing.

IV. CONCLUSION

Soursop incorporated stirred yoghurt is a beneficial option to carry nutritional and functional benefits to the consumers.

Addition of soursop extract results in boosting the antioxidant effect in the yoghurt product although significance difference was not observed in SJ and SN for its functional properties. SNY having good soursop flavor and reducing YMC and development of texture are recommended.

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