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Preparation of antioxidant rich low fat black grapes frozen yoghurt

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SUMMARY :

Black grapes fruit frozen yoghurt is the fermentation with *Lactobacillus bulgaricus* (Subsequently *L. bulgaricus*) and *Streptococcus salvarius* ssp. Culture bacteria apparently must be alive to provide benefits in the intestine tract. Black grapes fruit frozen yoghurt in the benefits of health effect promoted by consumption of grapes product are attributed to antioxidant rich and Polyphenol compound. As the largest group of polyphenols, flavonoid are the main candidates considered to have biological properties including antioxidant, anti-inflammatory, anti cancer, anti microbial, hypertension cardiovascular disease, obesity caused by free radical in the body which take up position in the body and compromises body's ability to resist foreign antigen further attacks health with the introduction of the antioxidant rich black grapes fruit frozen yoghurt.

KEY WORDS : Antioxidant, Black grapes, Yoghurt

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Yoghurt is regarded to be nutritious than the milk from which is made. Consumption of yoghurt provides energy through fat and carbohydrates, muscle building protein, bone forming minerals and essential growth factors is terms of vitamins through the action of micro-organism.

Yoghurt has been derived from a Turkish word "jugurt" that means to be curdled coagulated product obtained from pasteurized or boiled milk by lactic acid fermentation through *Lactobacillus bulgaricus* and *Streptococcus thermopiles.* It may contain culture of *Bifido bacterium bifidus, Lactobacillus acidophilus* and if added, the declaration to this shall be made on the label. Cultured milk product, particularly yoghurt, is regarded by many as health foods. They have a therapeutic significance particularly with reference to suppression of undesirable bacteria in the human digestive system. The product should have a uniform smooth body, texture characteristic and pleasing flavour with minimum whey separation. Weerathilake *et al.* (2014) Frozen yoghurt, including a minimum titratable acidity. The labeling regulation based on milk fat content the same as for ice

cream pH range of 5.76 to 6.72 and a titratable acidity (expressed as lactic acid) range of 0.20 to 0.43. In general, industry practices to achieve a minimum titratable acidity of 0.30 per cent with a minimum of 0.15 per cent titratable acidity resulting from fermentation bacteria. This titratable acidity may be achieved by fermentation with a mixture of *Lactobacillus delbruekii* spp. *Bulgaricus* (subsequently *L. bulgaricus*) and *Streptococcus salvarius* ssp. *Thermopiles* (subsequently, S.) *Thermopilus.* Culture bacteria apparently must be alive to provide benefits in the intestinal tract. Yoghurt bacteria survive gastrointestinal tract passage retention of the usefulness of lactic acid bacteria, it is necessary not only to promote its growth but also inhibit death of its cells further; it is required to maintain a high viable cell count in the final product during storage. For the maintenance of these physiological effect at high levels, it is important to retain useful bacteria such as lactic acid bacteria, in the high viable a cell count as possible in a viable state and further to keep high the activity (acid producing ability).

Stabilizers reduce crystallization, hinder *Lactobacillus bulgaricus* and *Streptococcus* melting and improve the handling properties of frozen yoghurt *Thermophilus.* The four main variables in the yogurt composition of frozen yogurt are fat, sugar, acid and stabilizers also prevent the air bubbles from solids. Fat and total solids are the main determinants collapsing and promote good flavour release and hold of textural quality and sugar and acid are the main flavuoring compounds in dispersion. Each has contributors to flavour. In the preparation of soft serve particular effect on body, texture, meltdown and stability and hard frozen products, it is desirous to have a storage. Grapefruit is an excellent source of vitamin C, a vitamin that helps to support the immune system and helps reduce cold symptoms or severity of cold symptoms. Vitamin C also prevents the free radical damage that triggers the inflammatory cascade. As free radicals can oxidize cholesterol and lead to plaques that may rupture causing heart attacks or stroke, helps in promoting cardio-vascular health.

Vitamin C is a good antioxidant contributor in grapefruit juice followed by of vitamin A, B complex, E and K. Larger amounts of calcium, folate, phosphorus, and potassium are found. The nutritive phytonutrient of this voluptuous fruit-liminoids, flavonoids, lycopene and glucarates-help fight cancer and various diseases. This juicy fruit contains citric acid, natural sugars and essential oils like limonene, pinene and citral.

EXPERIMENTAL METHODS

This present investigation "Preparation of antioxidant rich and low fat yoghurt by using black grapefruit pulp, orange pulp and pomegranate seeds" was conducted in the Nutrition Research Laboratory of the Department of Foods and Nutrition, Ethelind School of Home Science, Sam Higginbottom Institute of Agriculture Technology and Sciences, Allahabad, U.P.

Procurement of raw materials :

– Milk was purchased from Student Training Dairy, Department of Dairy Technology, SHIATS, Allahabad.

– Fresh ripe oranges, black grapes and pomegranates seeds were purchased from local fruits market of Allahabad.

– Yogurt culture was purchased from National Collection of Dairy Culture, Dairy Microbiology Division of NDRI Karnal Haryana, India.

– Sugar was purchased from local market of Allahabad.

Site of experiment :

The present investigation was carried out in the Nutrition Research Laboratory of Foods and Nutrition Department, Ethelind School of Home Science and Research Laboratory of Warner School of Food and Dairy Technology Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad.

Preparation of black grapes pulp:

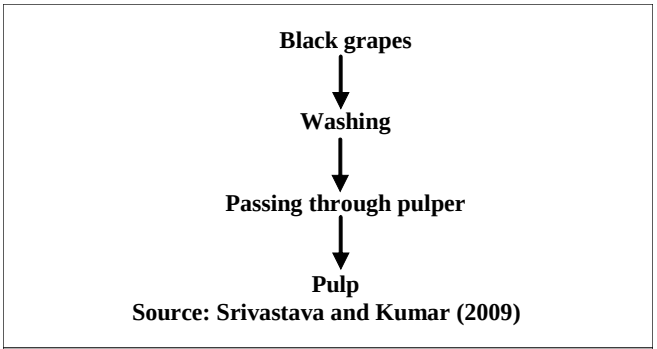

Fig. A : Flowchart for preparation of black grapes pulp

Treatment combination :

Treatments and replications of value added food products enriched with black grapes frozen yoghurt, orange frozen yoghurt, pomegranate yoghurt were as follows:

The basic frozen yoghurt added skimmed milk (80%), SMP (3%) sugar (14.6%), culture (2%), emulsifier (0.2%), stabilizer (0.2%) which served as control (T_0) for each product. The three value addition treatments were done with black grapes extract at (5%, 10%, 15%) level and referred to as T_1, T_2 and T_3, respectively. The amount of frozen yoghurt was varied at each treatment at 5 per cent, 10 per cent, and 15 per cent in accordance to all the three treatments. The amount of fruit 5 per cent, 10 per cent, 15 per cent for the products prepared, namely, black grapes frozen yoghurt. Control and treatments for each preparation were replicated 3 times, respectively.

Table A : Treatments of products

Control and treatments / Products	T_0	T_1	T_2	T_3
Skimmed milk	80%	75%	60%	65%
Sugar	14.6%	14.6%	14.6%	14.6%
Culture	2%	2%	2%	2%
Fruits	-	5%	10%	15%
Stabilizer	0.2%	0.2%	0.2%	0.2%
Emulsifier	0.2%	0.2%	0.2%	0.2%
SMP	3%	3%	3%	3%

Analysis of frozen yoghurt:

– Fat estimation by soxlet method (AOAC, 2007)

– Determination of ascorbic acid content – by 2-6 Dicloro endophenol.

– Calcium estimation by titration method (AOAC, 2007)

– Iron estimation using colourimeter (AOAC, 2007)

– Determination of total protein lowery method (AOAC, 1951)

– Determination of total carbohydrate content by anthrone method (AOAC,2007)

– Estimation of caloric value of energy value by calculation method (AOAC, 2007)

– Antioxidant activity was determined by DPPH method.

– Determination of total phenolic content.

– Determination of total flavonoids content.

Processing of frozen yogurt :

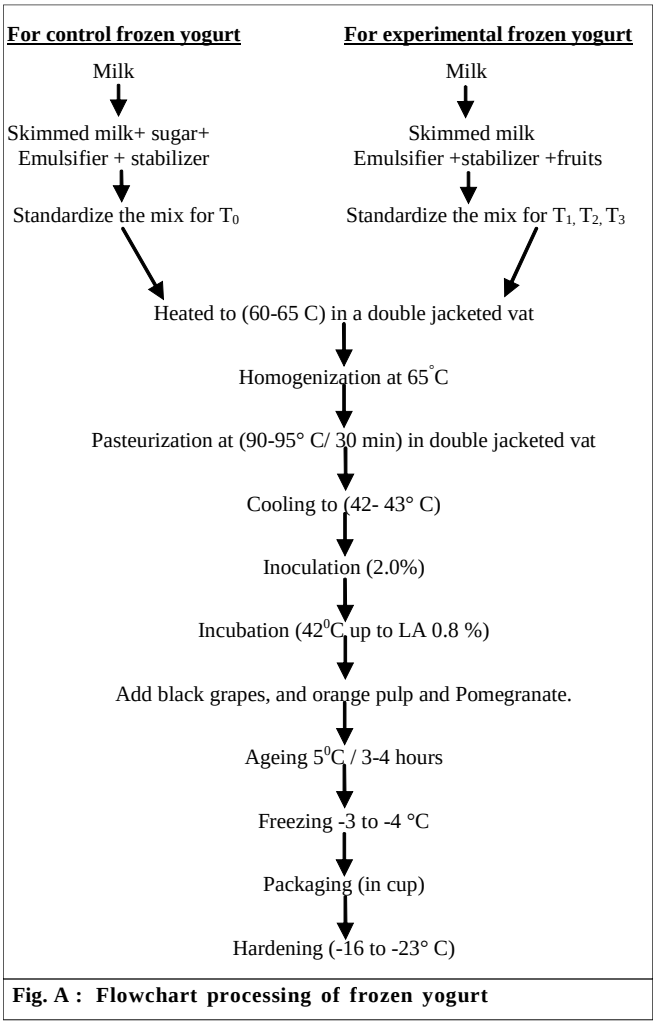

Fig. A : Flowchart processing of frozen yogurt

Analysis of sample :

Determination of total carbohydrate :

Principle :

Carbohydrates are first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green colored product with an absorption maximum at 630 nm.

Materials:

– 2.5 N HCl

– Anthrone reagent: 200 mg anthrone dissolved in 100 ml of ice-cold 95 per cent H_2SO_4. It should be prepared fresh before use.

– Standard glucose: Stock—Dissolve 100 mg in 100 ml water. Working standard—10 ml of stock diluted

to 100 ml with distilled water. Store refrigerated after adding a few drops of toluene.

Procedure :

– 100 mg of the sample was weighed into a boiling tube.

– It was hydrolyzed by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N HCl and cool to room temperature.

– It was further neutralized with solid sodium carbonate until the effervescence ceases.

– The volume was made to 100 ml and centrifuged.

– The supernatant was collected and 0.5 was taken and 1ml aliquots for analysis.

– The standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. '0' serves as blank.

– The volume was made to 1 ml in all the tubes including the sample tubes by adding distilled water.

– 4 ml of anthrone reagent was added.

– It was further heated for eight minutes in a boiling water bath.

– It was cooled rapidly and reading was taken from green to dark green colour at 630 nm.

– Standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.

– From the graph, the amount of carbohydrate present in the sample tube was calculated.

Calculation:

$$\text{Amount of carbohydrate present in 100 ml of the sample} = \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

Determination of vitamin C :

Principle:

This method was based upon the reduction of the dye 2-6-dichlorophenol indophenols by an acid solution of ascorbic acid. In the absence of interfering substances (Cu^{++}, Fe^{++}, Sn^{++} etc.) the reducing capacity of the extract of the sample is directly proportional to the ascorbic acid content.

Reagents:

– Standard ascorbic acid: 200 ml of pure ascorbic acid was accurately weighed on a tarred black glazed paper and transferred to a clean 100 ml volumetric flask. It was dissolved in 1 per cent oxalic acid solution and the volume was made upto the mark with same acid. Mixed well. The solution was immediately used as it was unstable. Water was not used as vitamin activity is lost when the lactone ring of dehydroascorbic acid is hydrolyzed by water to di-keto gluconic acid.

– 2-6-dichlorophenol-indophenol dye indicator: 50 mg of the sodium salt of 2-6-dichlorophenol-indophenol was dissolved or its equivalent in about 150 ml of hot water containing 42 mg of sodium bicarbonate. It was cooled under tap water and diluted to 200 ml with distilled water place in a brown colored glass stoppered bottle and was kept in a refrigerator or away from sunlight at 3^0C. The solution should not be keep for more than 1 week.

– Oxalic acid solution: 1 per cent.

Standardization:

The dye solution needs to be standardized every time it is used. 5 ml of the ascorbic acid standard solution was pipette out. In a small clean conical flask, 5 ml of 1 per cent oxalic acid solution was added and titrated with the dye indicator rapidly to a faint pink colour end point that persist for 15 sec. from the column of the dye used in the titration. The ascorbic acid equivalent of the dye was calculated in mg/ml.

Procedure:

– Yoghurt sample was taken and filtered through cheese cloth.

– 10ml of the juice was measured and pipette into a 100 ml volumetric flask and diluted to the mark with 1 per cent oxalic acid solution.

– It was mixed thoroughly. The dilute sample solution was filtered through dry filter paper.

– The first few ml of the filtrate was discarded.

– 10 ml or 20 ml aliquot of the filtrate was pipette into a small Erlenmeyer flask and titrated immediately with the standardized dye indicator solution to a faint pink colour end point that persist for 15 seconds.

Standardization of dye					
Sr. No.	Vol. of stan. Vit C	Conc. of stan. vit C	Initial vol of the dye (ml)	Final vol of dye (ml)	Difference (ml)
1.					
2.					
3.					

E = mg ascorbic acid/ ml of dye.

Determination of Vit C				
Sr. No.	Vol. of sample	Initial vol of dye(ml)	Final vol of dye(ml)	Difference (ml)
1.				
2.				
3.				

Calculation:

Calculate the ascorbic acid content in mg/100 ml of the sample as follows:

$$\text{Ascorbic acid mg/100 ml} = \frac{EV \times V \times 100}{V_2 \times W}$$

where,

E= Ascorbic acid equivalent of the dye in mg/ml

V= ml of the dye indicator used in the titration

V_1=Volume to which the yoghurt is diluted

V_2=Volume of the filtrate taken for the titration

W= Volume of the fruit juice initially taken for the determination

Result:

Ascorbic acid content of the sample was mg/ml.

Determination of antioxidant :

The yoghurt sample were filtered through 4-fold muslin cloth and the yoghurt was collected in clean containers.

Determination of total polyphenol content :

Principle :

Polyphenol was extracted with 70 per cent methanol from a test portion of finely ground sample at 70^0 C. The Polyphenol in the extract are determined calorimetrically using Folin-Ciocalteu phenol reagent. The reagent contains phosphor-tungstic acids as oxidants, which on reduction by readily oxidized phenolic hydroxyl groups yield a blue colour with a broad maximum absorption at 765nm. This is due to the formation of tungsten and molybdenum blues.

Procedures:

Standard solution- 0.110 g of gallic acid monohydrate (M= 188.14) was weighed into 100 ml volumetric flask. It was dissolve in water and diluted to the mark and mixed (stock standard). The volume of gallic acid stock standard solution given in (Table A) was transferred using pipettes to 100 ml one mark volumetric flasks. It was dilute to the mark with water and mixed. This dilute solution was prepared on the same day of used.

Table A : Gallic acid stock standard solution		
Gallic acid standard solution	Volume of gallic acid stock solution (ml)	Nominal concentration of dilute standard (µg/ml)
A	1.0	10
B	2.0	20
C	3.0	30
D	4.0	40
E	5.0	50

Sample preparation :

–5 ml of methanol was taken in test tubes (duplicates) and heated in water bath set at 70 ^{0}C and allowed at least 30 min for heating.

– 0.2 ml of samples (duplicates) was taken and dissolved in above test tubes. Heating was continued in water bath for 10 min.

– Tubes were removed from the water bath and allowed it to cool to room temperature.

– The supernatant of the two test tubes was carefully merged in another test tube.

– Using a pipette, 1 ml of the diluted sample extract was transferred into another test tube. Using a pipette, add 5 ml of dilute Folin-Ciocalteu phenol reagent into each test tube and mixed.

– Within 3 to 8 min after the addition of the diluted Folin-Ciocalteu phenol reagent, 4ml sodium carbonate solution was pipette into each test tube and was mixed carefully (blue colour appears).

– Allowed to stand at room temperature for 60 min and the optical density was measured by spectrophotometer set at 765nm.

Calculation :

Total content of polyphenolic compounds was calculated by the following formula :

$$X = \frac{5.6450 \times A}{m}$$

where,

X – Total polyphenolic compounds [%],

A – Absorbance,

m – Mass of investigated sample [ml].

Determination of total flavanoid content :

Reagents :

Aluminums trichloride, quercetin, ethanol

Procedure :

1 ml of 2 per cent aluminium trichloride was mixed

Table 1 : Average percentage of nutrients in control and treated samples of "Black grapes frozen yoghurt"

Control and treatments / Nutrients	T_0	T_1	T_2	T_3
Energy (kcal)	56	81.45	82.9	96.3
Carbohydrate (g)	7.4	8.54	9.68	10.82
Fat	1.55	1.58	1.61	1.63
Protein (g)	4.8	5.30	5.81	6.31
Vitamin C (mg)	2.2	9.5	19	28.5
Calcium (mg)	162	164.15	164.30	164.45
Iron (mg)	-	0.0135	0.027	0.0405
Total polyphenol content (mg)	4.5	110.4	175.8	281.2
Total flavonoid content (mg)	3.95	26.6	34.2	41.8

with the same volume of sample juice. Absorbance readings at 430nm were taken after 10 minutes against a blank sample consisting of 1 ml of sample solution and 1 ml of distilled water without aluminium trichloride. The total flavanoid content was determined using a standard curve of quercetin at 0-50 mg/ml. The average of three readings was used and then expressed as milligrams of quercetin equivalents/100 ml of juice sample.

Calculation :

Flavanoid content = quercetin equivalent (µg/ml) x total volume of ethanol extract (ml) + sample weight (ml) x dilution factor x 10^{-6} (g/µg) x 100

EXPERIMENTAL FINDINGS AND ANALYSIS

The data of the present studies "Preparation of antioxidant rich low fat Black grapes frozen Yoghurt" on different aspects as per the methodology was tabulated and analyzed statistically. The results

Nutritional composition of the products :

The Table 1 shows the nutritional composition of the frozen yoghurt skimmed milk (80%), SMP(3%), sugar (14.6%), emulsifier (0.2%), culture (2%), stabilizer (0.2%) as the control T_0. It has an appreciable amount of energy and carbohydrate. The vitamin C content is good. In the treated samples, where black grapes pulp has been incorporated along with skimmed milk (175%), SMP (3%), sugar (14.6%), culture (2%), emulsifier (0.2%), stabilizer (0.2%) has been added to all the treated sample pomegranate seeds as T_1 (5%), T_2 (10%), T_3 (15%) .

The Table 1 shows that average nutritional composition of black grapes frozen yoghurt with incorporation of black grapes shows that the nutrient content *i.e.* Energy, carbohydrate, protein, calcium, iron, Polyphenol, flavonoid increased with the addition of black grapes.

Summary :

The nutrient calculation of prepared frozen yoghurt (black grapes frozen yoghurt, orange frozen yoghurt, pomegranate frozen yoghurt) showed that the energy, protein, carbohydrate, iron, calcium, Polyphenol, flavanoid) content of the prepared frozen yoghurt were incorporation by fruits increased all the incorporation level of in all frozen yoghurt

Conclusion :

It is concluded that Skimmed milk fruit Black grapes is a rich source of antioxidant rich, calcium, energy, carbohydrate, vitamin C, Polyphenol and Flavonoids, can be successfully incorporated in the preparation black grapes frozen yoghurt showed that the treatment T_3 (Skimmed milk+ black grapes pulp+ sugar+ culture+ stabilizer+ emulsifier) was most acceptable and in case of Orange frozen yoghurt the treatment T_3 (orange pulp+ skimmed milk+ stabilizer + emulsifier +sugar) was the most acceptable of the prepare product in all treatment increased as the incorporation level was increased in all food product in as well as improve their Nutritional content.

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