Effect of flaxseed oil and flour on sensory, physicochemical and fatty acid profile of the fruit yoghurt

S. Siva Kumar^{1,2} · B. V. Balasubramanyam¹ · K. Jayaraj Rao¹ ·
P. Heartwin Amala Dhas¹ · B. Surendra Nath¹

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Abstract The objective of this study was to incorporate flaxseed oil, flaxseed flour and fruits for the preparation of fruit yoghurt and analysed for their sensory, physicochemical and fatty acid profile. Overall acceptability of sensory scores indicates that fruit yoghurt (20% fruit and sugar mixture) with incorporation of flaxseed oil up to 2% and flaxseed flour up to 1% in combination can be used for preparation of fruit yoghurt with acceptable sensory attributes. The scores drastically reduced for yoghurt samples wherein 2% flaxseed flour was incorporated. The mixture of sugar and fruits diluted the milk composition and resulted in lower values in the fruit yoghurts. The increase in fat, protein, carbohydrate, minerals content in the yoghurt was mainly contributed by the flaxseed oil and flour. The addition of flaxseed oil did not show any affect on the water activity of the yoghurt samples. The yoghurt had dietary fiber content of 1.24%. The fresh control fruit yoghurt showed the acidity value of 0.88% LA, while the corresponding value for experimental samples varied between 0.85 and 0.88% LA. TBA values increased with increased incorporation of flaxseed oil and flaxseed flour. The fatty acid analysis revealed that alpha linolenic acid (ALA) content increased from 0.45% of ALA in control sample to 22.80% of ALA in the final product.

Keywords Fruit yoghurt · Flaxseed oil · Flaxseed flour · Sensory · Physicochemical · Fatty acid profile · ALA

Abbreviations

FY	Fruit yoghurt
FY1/1	Fruit yoghurt with 1% flaxseed oil and 1% flaxseed flour
FY1/2	Fruit yoghurt with 1% flaxseed oil and 2% flaxseed flour
FY2/1	Fruit yoghurt with 2% flaxseed oil and 1% flaxseed flour
FY2/2	Fruit yoghurt with 2% flaxseed oil and 2% flaxseed flour
ALA	Alpha-linolenic acid (ALA)

Introduction

The growing awareness amongst consumers regarding several ingredients and their associated health benefits is making them more inclined to use nutritional information very often to make product choices. Hence there is a demand for food and beverages with health benefits and consumers are making a conscious attempt to include them in their diet. Milk is a mixture of specific bioactive proteins, lipids (saturated and unsaturated fatty acids), lactose, and minerals. The omega 3 fatty acid content in milk fat is less than 0.5% and alpha-linolenic acid is the main fatty acid in it (Jensen 2002). Omega-3s are one group of polyunsaturated fatty acids (PUFA) needed for optimal health. The World Health Organisation (WHO) and Food and Agriculture Organisation (FAO) Expert Committee recommended the omega-6 to omega-3 fatty acids ratio to be below 4 to prevent 70% of deaths caused by cardiovascular diseases (Simopoulos 2008). In the typical diet the

✉ S. Siva Kumar
drshiva2003@yahoo.com

¹ Southern Regional Station, National Dairy Research Institute, Bangalore, Karnataka 560030, India

² College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 141001, India

ratio of omega-6 to omega-3 essential fatty acids is as high as 15:1–16.7:1 (Carter 1993). This high omega-6/omega-3 ratio leads to many diseases, including cardiovascular disease, cancer, depression, and inflammatory and autoimmune diseases. On its own, the human body cannot make omega-3 fatty acids and so they must be obtained from food or supplements. As more consumers become aware of the potential therapeutic benefits of omega-3 fatty acids on the attenuation of chronic inflammation, an increased interest and demand for food and beverage products containing omega-3 fatty acids is increasing. Yoghurt, popular fermented milk product *has* excellent source of calcium and protein and poor source of omega-3 fatty acids and iron. Therefore, dairy products can be logical vehicles for omega-3 fatty acids and iron fortification, because they are widely consumed by all categories of population and also help in lowering the omega-6 to omega-3 fatty acid ratio.

The current scenario is that many food products including dairy products utilize marine-derived omega-3 fatty acids, which may develop a fishy or metallic off-flavour due to lipid oxidation problem (Let et al. 2005). In addition, many vegetarian and non-seafood consumers may be opposed to consuming these food products. A less explored alternative is the use of plant-derived omega-3 fatty acids in the formulation of novel functional food and beverage products. Flaxseed is the richest plant source of omega-3 fatty acids, fiber and lignans. The formulation of flaxseed-fortified milk products may provide a delivery vehicle for the combined nutrients found in milk and flaxseed. Flaxseed provides protein (30–35%), oil (30–45%) including omega-3 fatty acids, carbohydrates (30–35%), fiber (10%) and lignans (Bhatty 1995). Flaxseed components like lignans and ALA (Alpha Linolenic acids) may prevent tumour growth and reduce cancer risk in humans, lower blood cholesterol levels, reduce the risk of heart attacks and stroke, partly through the actions of ALA. Lignans are phytoestrogens (plant compounds), having estrogen-like actions in animals and humans. ALA may be especially important to vegetarians and people with low intakes of fatty fish. These characteristics make flaxseeds an attractive source of functional ingredients for the preparation of food products (Rubilar et al. 2010). Various forms of flaxseed that are available in the consumer food market include whole flaxseeds, milled flax, roasted flax and flax oil.

Sapota, one of the important tropical fruits belonging to the family Sapotaceae, popularly known as chikku fruit is a good source of digestible sugar, composes of soft, easily digestible pulp made of simple sugars like fructose and sucrose (12–20%), iron (0.8 mg) and calcium (21 mg) per 100 g (Shanmugavelu and Srinivasan 1973). Raisins (dried grapes) are *richer* sources of vitamins, minerals, energy

(100 g provide 249 calories) and poly-phenol anti-oxidants than the fresh grapes. Dates are a good source of dietary fiber (4.4–11.4%) depending on date variety and ripening stage (Spiller 1993).

In the present study, attempts were made to incorporate flaxseeds and fruits in yoghurt preparation, and study the effect of flaxseeds and fruits on sensory, physico chemical and fatty acid profile of fruit yoghurt. The incorporation of flaxseeds and fruits like dates, raisins, sapota (chikoo) is expected to improve the functional qualities of yoghurt through addition of omega-3 fatty acids and other nutritive content.

Materials and methods

Sugar/fruits/additives

The refined clean white crystalline sugar was procured from local market and used in preparation of fruit yoghurt. Selected fruits such as sapota, dates, and raisins were obtained from the local market. The sapota was washed under running water before it was used in the experiments. Deseeded dates of Lion brand obtained from the local market and raisins obtained from local market were cleaned for extraneous matter, washed in distilled water before they were used in the experiments.

Yoghurt cultures consisting of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, maintained in the Dairy Bacteriology section of the National Dairy Research Institute, Bangalore were used in the preparation of yoghurt. The cultures were subcultured 1 day before their use in the experiments.

Optimization of fruit and sugar mixture for preparation of fruit yoghurt with incorporation of flaxseed oil and flour

The yoghurt was prepared as per the procedure described by Tamime and Deeth (1980) with slight modifications for incorporation of flaxseed oil, flaxseed flour and fruits. The nutritional guidance value and the sensory attributes were considered for conducting the preliminary trials. Based on the sensory evaluation, incorporation of flaxseed flour up to 2%, and flaxseed oil up to 2% were selected for further trials. At the end of 4 h of incubation, the yoghurt has shown the acidity of 0.76% which was ideal acidity to be shifted to cold store (about 5 °C). Based on the higher content of iron, sapota, dates and raisins (as per the available literature) were selected for preparation of fruit yoghurt. After cleaning, the fruits were cut into small pieces and mixed in equal proportions. Predetermined level of

sugar was added to the mixture and stored at refrigerated temperature for overnight before the mixture was used for the preparation of fruit yoghurt. When equal proportions of fruits were used in the mixture, the fruit mixture flavour masked the delicate yoghurt flavour and the fruit flavour was found dominated in the fruit yoghurt. After trials on different proportions of the fruits, it was concluded that two parts of raisins, three parts of dates and five parts of sapota with 10% sugar resulted in obtaining fruit flavoured yoghurt with delicate yoghurt flavour. To optimize the level of mixing of fruit-sugar mixture with yoghurt, 15, 20 and 25% of the fruit-sugar mixture was added to previously prepared yoghurt, filled in polystyrene cups and stored for overnight for equilibration of the fruit and yoghurt flavours in the product and served to a panel of judges for sensory evaluation. The sensory evaluation of the experimental yoghurt samples was carried out with the help of 9 point hedonic scale (Shone et al. 1977).

Physicochemical analysis of fruit yoghurt

Total Solids (Gravimetric method), Fat (Mojonnier Fat-Extraction apparatus), Total Protein (Micro Kjeldahl method) and Ash contents were estimated by AOAC (2006).

Total Carbohydrate content was calculated by difference method. The total calorific content (kcal) was estimated by the formula method (Gopalan et al. 1989).

Titrateable acidity

Titrateable acidity of the yoghurt samples was estimated according to the standard AOAC procedure (Helrich 1990).

Degree of syneresis

The syneresis of the yoghurt expressed as free whey, was measured by method used by Al-Kadamany et al. (2003).

Dietary fiber estimation

Total dietary fiber content was analyzed as per the procedures given in Megazyme ‘Total Dietary Fiber Assay Kit’ (K-TDFR 05/12) supplied by Megazyme International Ireland 2012, supplied in India by Pro Lab Marketing Pvt. Ltd, New Delhi).

Water activity

The water activity (aw) of the product was measured using the digital water activity meter (Rotronic, Switzerland).

TBA value

The oxidative changes in the control and experimental yoghurts were measured in terms of thiobarbituric acid value (TBA) (King 1962). It was expressed as optical density (OD) at 532 nm.

Fatty acid profile analysis of yoghurt

The effect of processing conditions on omega 3 fatty acids content in the product was studied at the end of fermentation by using gas liquid chromatography (GLC) as per ISO procedures mentioned below:

ISO 15885 | IDF 184, 2002, ISO: 14156 IDF | 172 and ISO/DIS (23065).

Lipid extraction and preparation of fatty acid methyl esters (FAME)

Fatty acid composition was determined by gas chromatography after derivatization to fatty acid methyl esters (FAME). The preparation of FAME was performed according to the standard method, with additional alterations in the separation and filtration process and time.

GLC analysis of FAME

Quantification of FAME in samples was performed using gas chromatograph (Chemito, GC-8610) equipped with flame ionization detector (FID) and computer assisted data processing. The methyl esters of various fatty acids were separated using C18 capillary column (Supelco). The operating parameters were: column details—length: 30 M; diameter: 0.32 mm; film thickness: 0.25 mm. Injection was performed at split mode (1:20). Oven temperature details—initial temperature: 160 °C for 3 min, increased at the rate of 5 °C/min followed by 20 °C/min to a final temperature of 220 °C and held for 3 min. Injection port temperature was 230 °C and detector temperature was 240 °C. High purity nitrogen gas was used as a carrier gas at a constant 5 psi pressure with flow rate of 3–5 ml/min. Flame ionization detector was supplied with hydrogen (30 ml/min) and air (300 ml/min). The fatty acids were identified by comparing the retention times of fatty acids methyl esters standard mixture and calculated by area percent method through the integrated software package given by the manufacturer. Area counts of peaks were evaluated to estimate proportion of saturated, monounsaturated and polyunsaturated fatty acids present in the product. Averages of fatty acid as percent of total lipid weight of its respective samples are reported. Values are expressed as fatty acid content (% on fat basis) of yoghurt.

Sensory evaluation

The organoleptic quality of yoghurt samples were evaluated by eight trained panellists drawn from the faculty and students of the Dairy Technology Division, National Dairy Research Institute, Bangalore. The yoghurt samples (30–40 g) were served at 5 °C and analysed for sensory parameters like, colour and appearance, flavour, body and texture and overall acceptability using nine point hedonic scale (Shone et al. 1977) wherein a score of one represented “dislike extremely” and score of nine represented “like extremely”. The samples for evaluation were coded appropriately before serving the samples to the judges for sensory evaluation. One day Training programme was conducted with the aim/title on sensory evaluation of fruit yoghurt incorporated with flaxseed oil and flour. Sensory evaluation of the samples was carried out in the sensory evaluation room under appropriate fluorescent lighting. Between evaluations of the samples the panellists rinsed their mouths with de-ionised water to remove any lingering sweet taste. The tests were carried out 1 h before or 2 h after the mid day meal.

Statistical analysis

Sensory, Physicochemical and fatty acid profile data of the samples obtained from various experiments were statistically analysed by Analysis of Variance using statistical software SPSS 15.0 for Windows Evaluation Version. A probability level of $P \leq 0.05$ was used for testing the statistical significance of all experimental data.

Results and discussion

The fruit yoghurt prepared by incorporating flaxseed oil and flour was analysed for their sensory, physicochemical and fatty acid characteristics and the findings are presented in Tables 1 and 2 respectively. Toned milk was used in the preparation of control and experimental yoghurt. The SNF content of the milk was adjusted to 10% before using it in the experiments. During the preliminary trials the sugar level was standardized. It was found that use of 10% sugar for preparation of flaxseed flour added yoghurt was optimum. Based on, iron content (as per the available literature), dates, raisins and sapota fruits were selected for incorporation in yoghurt. The fruit-sugar mixture was added to yoghurt at 15, 20 and 25% level and served to a panel of judges for sensory evaluation. The scores are presented in Table 1.

Sensory analysis of fruit yoghurt

Colour and appearance

The scores show that the control fruit yoghurt with 15% fruit-sugar mixture scored a score of 8.36. The sapota fruit imparted light and pleasant brown colour to the product. The score reduced to 8.19 and 8.17 for the FY1/1 and FY2/1 yoghurts having 1% flaxseed flour. The scores reduced significantly to 7.4 and 7.34 for FY1/2 and FY2/2 yoghurt having 2% flour. The addition of 2% flour resulted in darker brown colour with more amount of flaxseed particles appearing on the surface of product. The statistical analysis indicates that the yoghurt with 1% flour and 2% flour were significantly different from the control yoghurt. Addition of 20% and above amount of fruit-sugar mixture increased the intensity of brown colour resulting in lower score for both control and experimental samples, this could be due to brown colour imparted by increased amounts of sapota and dates. Balasubramanyam and Kulkarni (1991) reported that colour and appearance decrease may be due to mango pulp particles settling at the bottom in set yoghurt at higher mango pulp concentrations and increased wheying off in case of addition of excess mango, strawberry and pineapple in the yoghurt. The colour and appearance of control and experimental yoghurt samples varied significantly ($P \leq 0.05$) while no significant variation was observed between FY1/1 and FY2/1, indicating that higher flaxseed oil incorporation did not affect the colour and appearance.

Body and texture

The body and texture score indicated that the control yoghurt with 15% mixture scored an average score of 7.50 and the score reduced to 7.40 and 7.29 respectively for 20 and 25% mixture added yoghurt. The consistency of the yoghurt became thinner with increased level of the mixture. The milk solids level which was contributing for the body and texture, decreased with increased proportion of the fruit-sugar mixture which could be responsible for the reduced consistency. The scores for 1% flaxseed flour incorporated yoghurt samples (FY1/1 and FY2/1) slightly improved to 7.59 and 7.58 respectively compared to control samples. The mucilaginous substances present in the flaxseed flour have improved the consistency and the products appeared slightly thicker compared to control. As in the case of control, the scores reduced with increased addition of the fruit-sugar mixture. However, the drop in scores was slightly less compared to control due to improved consistency. The presence of higher level of flaxseed flour i.e. 2% in yoghurt for FY1/2 and FY2/2 the scores reduced to 6.96 and 6.83 for 15% fruit-sugar

Table 1 Sensory scores of Fruit Yoghurt with incorporation of flaxseed oil and flour

Sensory parameters	Fruit sugar mixture (%)	FY (control)	FY1/1	FY1/2	FY2/1	FY2/2
Colour and appearance	15	8.36 ^{aA} ± 0.11	8.19 ^{aB} ± 0.18	7.40 ^{aC} ± 0.14	8.17 ^{aB} ± 0.10	7.34 ^{aC} ± 0.09
	20	8.30 ^{aA} ± 0.03	8.17 ^{aB} ± 0.18	7.30 ^{bC} ± 0.17	8.13 ^{aB} ± 0.18	7.20 ^{bD} ± 0.11
	25	7.94 ^{bA} ± 0.06	7.90 ^{bB} ± 0.07	7.21 ^{cC} ± 0.13	7.83 ^{bB} ± 0.13	7.11 ^{cD} ± 0.09
Body and texture	15	7.50 ^{aB} ± 0.11	7.59 ^{aA} ± 0.12	6.96 ^{aC} ± 0.05	7.58 ^{aA} ± 0.07	6.83 ^{aC} ± 0.13
	20	7.40 ^{bB} ± 0.13	7.52 ^{bA} ± 0.13	6.83 ^{bC} ± 0.17	7.53 ^{bA} ± 0.05	6.81 ^{aC} ± 0.12
	25	7.29 ^{cB} ± 0.20	7.42 ^{cA} ± 0.15	6.69 ^{cC} ± 0.16	7.36 ^{cA} ± 0.10	6.61 ^{bC} ± 0.11
Flavour	15	8.25 ^{bA} ± 0.14	8.15 ^{bB} ± 0.13	7.17 ^{bC} ± 0.12	8.12 ^{bB} ± 0.10	7.08 ^{bC} ± 0.14
	20	8.36 ^{aA} ± 0.09	8.28 ^{aA} ± 0.10	7.38 ^{aB} ± 0.09	8.25 ^{aA} ± 0.08	7.31 ^{aB} ± 0.10
	25	7.94 ^{cA} ± 0.07	7.88 ^{cB} ± 0.05	7.01 ^{cC} ± 0.09	7.79 ^{cB} ± 0.05	6.93 ^{cC} ± 0.08
Overall acceptability	15	8.08 ^{aA} ± 0.10	8.00 ^{bB} ± 0.05	7.10 ^{bC} ± 0.17	7.95 ^{bB} ± 0.10	7.05 ^{bC} ± 0.15
	20	8.11 ^{aA} ± 0.15	8.05 ^{aA} ± 0.09	7.26 ^{aB} ± 0.10	8.01 ^{aA} ± 0.11	7.20 ^{aB} ± 0.18
	25	7.70 ^{bA} ± 0.09	7.70 ^{cA} ± 0.10	7.01 ^{cB} ± 0.14	7.68 ^{cA} ± 0.08	6.89 ^{cB} ± 0.13

Mean values with different Superscript ^{ABCD} in a row/^{abc} in a column differ significantly at $P \leq 0.05$

Statistical mean ± SD (n = 6)

FY fruit yoghurt, FY1/1 fruit yoghurt with 1% flaxseed oil and 1% flaxseed flour, FY1/2 fruit yoghurt with 1% flaxseed oil and 2% flaxseed flour, FY2/1 fruit yoghurt with 2% flaxseed oil and 1% flaxseed flour, FY2/2 fruit yoghurt with 2% flaxseed oil and 2% flaxseed flour

Table 2 Physicochemical and fatty acid characteristics of fruit yoghurt prepared by incorporation of flaxseed oil and flour

Parameters (%)	FY (control)	FY1/1	FY1/2	FY2/1	FY2/2
<i>Physicochemical characteristics</i>					
Total fat	2.41 ^a ± 0.01	3.72 ^b ± 0.02	4.03 ^c ± 0.02	4.66 ^d ± 0.02	5.06 ^e ± 0.02
Protein	3.23 ^a ± 0.04	3.46 ^b ± 0.02	3.53 ^c ± 0.02	3.42 ^b ± 0.02	3.51 ^c ± 0.02
Total carbohydrate	23.00 ^a ± 0.02	23.23 ^a ± 0.02	23.55 ^c ± 0.01	23.33 ^b ± 0.01	23.66 ^c ± 0.02
Minerals	0.85 ^a ± 0.01	0.89 ^b ± 0.01	0.92 ^c ± 0.01	0.89 ^b ± 0.01	0.92 ^c ± 0.01
Total solids	30.48 ^a ± 0.02	32.54 ^b ± 0.02	33.03 ^c ± 0.01	33.54 ^d ± 0.02	34.59 ^e ± 0.02
Energy (kcal)	126.61 ^a ± 0.02	140.24 ^b ± 0.01	144.59 ^c ± 0.01	148.94 ^d ± 0.02	154.22 ^e ± 0.01
Dietary fiber	0.99 ^a ± 0.02	1.24 ^b ± 0.01	1.46 ^c ± 0.02	1.24 ^b ± 0.02	1.44 ^c ± 0.01
aw	0.966 ^{bc} ± 0.001	0.963 ^b ± 0.001	0.953 ^a ± 0.001	0.964 ^b ± 0.001	0.952 ^a ± 0.001
T. Acidity (%LA)	0.88 ^a ± 0.04	0.87 ^a ± 0.02	0.86 ^b ± 0.04	0.88 ^a ± 0.01	0.85 ^b ± 0.01
pH	4.23 ^a ± 0.02	4.23 ^a ± 0.04	4.25 ^b ± 0.01	4.22 ^a ± 0.01	4.25 ^b ± 0.02
Syneresis (ml/10 g sample)	0.67 ^c ± 0.02	0.62 ^b ± 0.01	0.60 ^a ± 0.04	0.62 ^b ± 0.02	0.57 ^a ± 0.01
TBA (OD 532 nm)	0.027 ^a ± 0.02	0.047 ^b ± 0.01	0.052 ^c ± 0.02	0.058 ^d ± 0.01	0.063 ^e ± 0.02
<i>Fatty acid profile (values are expressed as fatty acid content (% on fat basis) of yoghurt)</i>					
Myristic acid	10.30 ^c ± 0.02	6.28 ^b ± 0.01	6.35 ^b ± 0.03	6.51 ^b ± 0.02	5.28 ^a ± 0.01
Palmitic acid	30.95 ^c ± 0.03	21.06 ^b ± 0.01	20.85 ^b ± 0.04	20.08 ^a ± 0.01	19.06 ^a ± 0.01
Stearic acid	12.72 ^d ± 0.03	9.53 ^c ± 0.04	8.24 ^b ± 0.03	8.17 ^b ± 0.04	7.53 ^a ± 0.02
Oleic acid	28.01 ^d ± 0.02	24.72 ^c ± 0.02	24.55 ^c ± 0.01	23.75 ^b ± 0.03	22.82 ^a ± 0.01
Linoleic acid	3.40 ^a ± 0.02	5.54 ^b ± 0.03	5.76 ^b ± 0.04	6.26 ^c ± 0.01	6.83 ^c ± 0.04
Alpha linolenic acid	0.45 ^a ± 0.01	18.25 ^b ± 0.04	18.40 ^b ± 0.03	22.80 ^c ± 0.02	23.12 ^c ± 0.03
Palmitoleic acid	1.01 ^b ± 0.04	1.15 ^b ± 0.02	1.26 ^c ± 0.01	0.66 ^a ± 0.02	1.21 ^c ± 0.01
Others	13.16 ^b ± 0.01	13.47 ^b ± 0.03	14.59 ^c ± 0.02	11.77 ^a ± 0.04	14.15 ^c ± 0.01

Mean values with different Superscript ^{abcde} in a row differ significantly. $P \leq 0.05$ (mean ± SE) n = 6

FY fruit yoghurt, FY1/1 fruit yoghurt with 1% flaxseed oil and 1% flaxseed flour, FY1/2 fruit yoghurt with 1% flaxseed oil and 2% flaxseed flour, FY2/1 fruit yoghurt with 2% flaxseed oil and 1% flaxseed flour, FY2/2 fruit yoghurt with 2% flaxseed oil and 2% flaxseed flour

mixture added yoghurts since the products became stickier. As in the case of control and yoghurt with 1% flour, the scores reduced with increased addition of the mixture, due to the reduced consistency. Goyal et al. (2016) reported that the scores for body and texture of fortified dahi decreased significantly with increase in the level of WPC encapsulated Microencapsulated flaxseed oil powder (MFOP) preparations from 2 to 3%. Palmer (1971) observed that banana pulp addition in fruit dahi increased the firmness due to stabilizing effect of starch and pectic substances present in the banana pulp and similar phenomena was observed in sapota fruit yoghurt. Ramasamy and Basak (1992) reported that use of pectin in stirred fruit yoghurt greatly increased the apparent viscosity and increasing pectin concentration significantly decreased whey separation and also prevented the sedimentation in cultured milk beverages. Vahedi et al. (2008) reported that the texture of yoghurt improved when apple and strawberry fruits were added to yoghurt after fermentation. The score revealed no significant difference between FY1/1 and FY2/1 yoghurts which scored better than control yoghurt; while other samples including control yoghurt significantly differed from these two samples ($P \leq 0.05$).

Flavour

The flavour scores for control fruit yoghurt with 15% fruit-sugar mixture scored an average score of 8.25. The product had good delicate yoghurt flavour complemented with delicate sapota and dates flavours. Since raisins were added in whole form the delicate raisins flavour was felt when the whole raisins were bitten in the mouth. The flavour score of the yoghurt improved to 8.36 when 20% of the mixture was used in the yoghurt. However, the flavour score reduced to 7.94 when 25% mixture was used in the fruit yoghurt. The delicate yoghurt flavour was found masked and the fruit flavour particularly of sapota dominated the flavour in the products. Microencapsulated flaxseed oil powder fortified dahi samples at 3% level showed significantly lower flavor scores as compared to control may be due to excess amount of flaxseed oil present in the product and also may be due to release of encapsulated flaxseed oil during pasteurization/fermentation process (Goyal et al. 2016). Vahedi et al. (2008) reported that mouth feel score of apple and strawberry fruit yoghurts was higher in samples in which fruit cubes were added after fermentation. Davis et al. (1978) and Balasubramanyam and Kulkarni (1991) reported that addition of fruits increased the flavour of the yoghurt. Proper fermentation imparts delicate flavour to the product. Pandhya (2002) reported that the sensory scores of yoghurt decreased with decrease in fat content. Addition of fruits increased the flavour depending on the type of fruits added. The texture scores decreased with increased pulp

concentrations. Further, he reported that addition 6% mango pulp, 4% strawberry, 8% pineapple resulted in production of fruit dahi with good sensory attributes. Use of 8% sugar was found to be optimum for production of fruit dahi. The statistical analysis revealed that the flavour of yoghurt containing 20% fruit-sugar mixture was significantly higher than others ($P \leq 0.05$). The score also revealed that significant difference was found between control and all experimental samples. The judges liked the FY1/1 and FT2/1 fruit yoghurt containing 1% flaxseed flour as compared with control.

Overall acceptability

The overall acceptability score of the control fruit yoghurt with 15% fruit-sugar mixture was 8.08. The scores reduced to 8.00 and 7.95 respectively for FY1/1 and FY2/1 yoghurts. Similarly, the scores reduced significantly to 7.10 and 7.05 for FY1/2 and FY2/2 yoghurts wherein 2% flaxseed flour was incorporated. The scores for these samples reduced mainly because of flavour and consistency. When 20% fruit-sugar mixture was used for fruit yoghurt preparation, the control fruit yoghurt scored an overall acceptability score of 8.11 which was marginally higher than that for 15% fruit-mixture incorporated yoghurt. Similarly the scores for FY1/1 and FY2/1 were 8.05 and 8.01 respectively which were slightly higher than those for 15% fruit-sugar mixture added yoghurt. The scores drastically reduced for FY1/2 and 2/2 yoghurt samples wherein 2% flaxseed flour was incorporated. When 25% fruit-sugar mixture was used in the yoghurt, the scores of all the yoghurt samples including control reduced when compared to those for 20% fruit-sugar added yoghurts. The scores revealed that among the different levels of fruit-sugar mixture tried, the use of 20% fruit –sugar mixture was better accepted than others, while among the different combinations of flaxseed oil and flaxseed flour, no significant difference ($P \leq 0.05$) was observed between control and FY1/1 and FY2/1 yoghurt samples. Similar kinds of information also reported by Goyal et al. (2016) that omega-3(microencapsulated flaxseed oil powder) dahi fortified at 2% level sensory scores were comparable with control. Cheddar cheese and mozzarella cheese were fortified with omega 3 from flaxseed oil (FO) and microencapsulated fish oil (MFO) at specific stages of cheese making process. Sensory evaluation (5 point hedonic scale) showed similar colour acceptability for all three cheeses regardless of omega source. However, scores ranged from 2.43 (sample fortified with MFO) to 3.6 (Control sample and one fortified with flaxseed oil) for aroma. Flavour of cheese fortified with flaxseed oil was preferred over MFO by panelists. Overall, omega 3 was an excellent nutrient that could be easily added to cheese with only minor

changes (Bermúdez-Aguirre and Barbosa-Cánovas 2011). Hashim et al. (2009) investigated the effect of fortification with date fiber (DF) and wheat bran (WB) on fresh yoghurt quality. Thus the study finally indicates that fruit yoghurt (20% fruit-sugar mixture) with incorporation of flaxseed oil @ 2% and flaxseed flour @ 1% in combination can be used for preparation of fruit yoghurt with acceptable sensory attributes and also keeping in view of its health benefits.

Physicochemical analysis of fruit yoghurt

Fat

The average fat content in the control yoghurt was 2.41%. Though the milk initially had 3.0% fat, the addition of culture (4% of milk taken), mixture of sugar and fruits has diluted the milk composition and resulted in lower values. The fat content in FY1/1 yoghurt was found to be higher at 3.72 compared to 2.41% in control yoghurt. The flaxseed oil and the lipid portion of the flaxseed flour have contributed for the higher fat in the yoghurt. Similarly the fat content in yoghurt FY2/1, FY2/1, and FY2/2 increased gradually to 4.03, 4.66 and 5.06 respectively. Daun et al. (2003) and Kozłowska (1989) reported that flaxseed contains 30–45% fat, the oil content in the flaxseed (38–45%) may vary depending on location, cultivar and environmental conditions. Further, Spiller (1993) and Shanmugavelu and Srinivasan (1973) reported that dates, raisins and sapota contribute minor amount of fat to food products.

Protein

The protein content in control yoghurt was 3.23% while the protein content in FY1/1 and FY2/1 was 3.46 and 3.42% respectively. Similarly the protein content in FY2/1 and FY2/2 were 3.53 and 3.51 respectively. The protein content in the yoghurt having higher flaxseed flour (FY1/2 and FY2/2) was higher, indicating that the increase in the protein content was mainly contributed by the flaxseed flour. Daun et al. (2003) and Kozłowska (1989) reported that flaxseed contained high protein content. Dates, raisins, and sapota might have contributed a minor amount of proteins in addition to flaxseed flour proteins.

Total carbohydrate content

The total carbohydrate content in control yoghurt was 23.00%. While in experimental yoghurt FY1/1 and FY1/2 the carbohydrate content was 23.23 and 23.33 respectively. The corresponding values for FY2/1 and FY2/2 were 23.55 and 23.66. In addition to the lactose from milk, sucrose from added sugar, and the carbohydrates from flaxseed flour and added fruits have contributed for the total

carbohydrates in the yoghurt samples. The slight increase in the carbohydrate content in the experimental yoghurts compared to that in control yoghurt was mainly due to the flaxseed carbohydrates. Within the experimental samples, it was observed that the yoghurt containing higher flour level (FY1/2 and FY2/2) showed slightly higher carbohydrate compared to lower flour containing yoghurt samples (FY1/1 and FY2/1), indicating that the flour is also contributing to the carbohydrates in the yoghurt.

Minerals

The total minerals content in control yoghurt was found to be 0.85%. Normally milk contains about 0.80% minerals while the control showed slightly higher minerals content, which could have been contributed by added fruits. Though, the milk solid in the fruit yoghurt was diluted by the addition of sugar and fruits, the fruits have shown higher mineral content. This clearly indicates the fruits that have been added have significantly contributed to the minerals in the yoghurt. As in the case of the proteins, experimental yoghurts have shown slightly higher minerals content, because flaxseed flour might have contributed to it. The yoghurt with 1% flaxseed flour (FY1/1 and FY2/1) showed the mineral content of 0.89% while the yoghurt with 2% flour (FY1/2 and FY2/2) showed slightly higher mineral content of 0.92% which substantiates the fact that the flour contributes to minerals in the products. The results are in accordance with the findings of Hussain et al. (2008), USDA National Nutrient data base (2015), Tarak and Dag (2013). Statistical analysis shows significant difference between the control and experimental yoghurt samples ($P \leq 0.05$).

Total solids

Total solids of the control sample was (30.48%) and the experimental samples ranged between 32.54 and 34.59%. Statistical analysis shows significant difference between the control and experimental yoghurt samples ($P < 0.05$) which may be due to addition of flaxseed powder and flaxseed oil.

Dietary fiber

Yoghurt is devoid of fiber and there is a need for fortification of dairy products to increase their functional quality. The type of fiber and its level of incorporation may affect the quality and acceptability of the dairy foods. In our studies, attempts were made to incorporate flaxseed flour and fruits in yoghurt. The control fruit yoghurt contained a fiber content of 0.99% which was wholly contributed by the added fruits. The fiber content in FY1/

1 yoghurt increased to 1.24% and further increased to 1.46% in FY1/2 yoghurt. Similar corresponding values were observed for FY2/1 and FY2/2 yoghurts. The study clearly indicated that the fiber content increased with increased incorporation of flaxseed flour since higher values were seen in yoghurts containing 2% flaxseed flour. Simmons et al. (2010) and Lakka et al. (2002) reported that flaxseed contains both soluble (one-third) and insoluble fiber (two-third) amounting to about 28 g total per 100 grams of the flaxseed. The results are in accordance with the findings of Mueller et al. (2010), Coskuner and Karababa (2007), Spiller (1993) and Shanmugavelu and Srinivasan (1973).

Water activity

The addition of flaxseed oil did not show any effect on the water activity of the yoghurt samples. The control yoghurt showed the water activity of 0.966 and the samples FY1/1 and FY2/1 yoghurt which had 1% flaxseed oil and 1% flaxseed flour showed the water activity of 0.963 and 0.964 respectively which were not significantly different from that of control yoghurt. Fajardo-Lira et al. (1997) reported that the fat has no role in a_w . They also reported that milk has a_w of 0.993 and that of plain unsweetened and sweetened yoghurts (12% sucrose and 10% milk solids) had a_w of 0.994 and 0.985 respectively indicating that addition of sugar to milk before fermentation reduces a_w . The FY1/2 and FY2/2 yoghurt which had 1% oil and 2% flour showed the water activity values of 0.953 and 0.952 respectively which were slightly less than the other yoghurt samples. The lower water activity values in 2% flaxseed flour yoghurt which could be due to binding of water by mucilaginous materials from flaxseed flour. The lower water activity in these samples has slightly affected the bacterial activity which can be seen from Table 2, where lower acidity development in these yoghurts was recorded. Hussain et al. (2006) reported that flaxseed is high in mucilage (gums) that can increase the water absorption properties resulting in reduced water activity.

Caloric value

The calorific value of the control and experimental samples are calculated by formula method mentioned by Gopalan et al. (1989). The control yoghurt had a calorific value of 126.61 kcal/100 g of product. The calorific values of the experimental samples increased with increased addition of flaxseed oil and flaxseed flour. The statistical analysis shows significant difference ($P \leq 0.05$) between control and experimental samples. The significant difference was due to addition of flaxseed oil and flaxseed flour.

Titrateable acidity and pH

The acidity values in flaxseed oil and flour incorporated fruit yoghurt are presented in the Table 2. The fresh control fruit yoghurt showed the acidity value of 0.88% LA, while the corresponding value for experimental samples varied between 0.85 and 0.88% LA. The rate of acidity development in flaxseed flour incorporated yoghurt was slow, at the end of overnight storage at 5 °C, the acidity in these samples reached to that of control and other experimental samples. Similarly the fresh control fruit yoghurt showed the pH value of 4.23, while the corresponding value for experimental samples varied between 4.22 and 4.25. Oakenfull (2001) and Lim et al. (2010) reported that the type of flaxseed fiber may be contributed to increase in the pH value of the samples, due to the reaction of the linked monosaccharide units, constitute the soluble fiber or polysaccharide. However, higher fiber levels were significantly ($P \leq 0.05$) associated with lower titrateable acidity in the experimental samples.

Syneresis

The fresh control fruit yoghurt has showed the syneresis of 0.67 ml per 10 g of the sample, while the corresponding values for FY1/1 and FY2/1 yoghurt were 0.62 ml. The syneresis values for FY1/2 and FY2/2 were 0.60 and 0.57 ml respectively. It was observed that addition of 1% flaxseed flour (FY1/1 and FY2/1 yoghurt) has reduced the syneresis compared to that in control yoghurt sample and the values further reduced in 2% flour incorporated (FY1/2 and FY2/2) yoghurt samples. The statistical analysis shows significant difference ($P \leq 0.05$) between control and experimental samples. The binding of water by the mucilage substances present in flaxseed flour might be the reason for reduced syneresis with increased level of flaxseed flour in the product. Diaz-Jimenez et al. (2004) prepared set and stirred yoghurts to analyze the effect of wheat bran fiber addition and low fat, and reported that the added fiber decreased the syneresis and increased the consistency.

TBA value

The TBA value of control containing fruit yoghurt was 0.027, while the experimental yoghurt samples showed higher TBA values ranging from 0.047 to 0.063. The values increased with increased incorporation of flaxseed oil and flaxseed flour. This indicates that both the flaxseed oil and the flour have contributed to the TBA value. The statistical analysis shows significant difference ($P \leq 0.05$) between control and experimental samples. Tong et al. (2000) and Kolanowski et al. (2007) reported that long

chain PUFA are very susceptible to oxidation. Moya et al. (1999) studied the thermal oxidation of polyunsaturated fatty acids and reported that a decrease in unsaturation occurred at 150 °C and above. Since the flaxseed oil is rich in PUFA and processing parameters such as pre-heating, homogenization and pasteurization also probably initiated the oxidation of the fatty acids in the experimental yoghurt samples.

Fatty acid profile of fruit yoghurt

The omega 3 fatty acids and other fatty acid content in the fruit yoghurts were estimated by GLC method and the results are presented in Table 2.

The control yoghurt showed ALA content of 0.45%, LA content of 3.40% and oleic acid 28.01%. The flaxseed oil and flour incorporated yoghurt had oleic acid in the range of 22.82–24.72%. The flaxseed oil and flour incorporated yoghurt had LA in the range of 5.54–6.83% and ALA in the range of 18.25–23.12 levels in the extracted fat. It is thus evident that supplementation of flaxseed oil and flours significantly ($P \leq 0.05$) increased the level of omega-3 fatty acids in the final product. Daun et al. (2003) and Kozłowska (1989) reported that oil content in flaxseed falls in the range 38–45% depending on location, cultivar, and environmental conditions. Flaxseed has a high ALA content, generally constituting 50–62% of total fatty acids.

Goh et al. (2006) claimed that the polyunsaturated fat (linoleic and α -linolenic acids) in flaxseed oil is about 80%. The most important fatty acids for human health are—C18:1, C18:2 and C18:3. A significant difference was found in the content of the polyunsaturated fatty acid C18:3 between control yoghurt and yoghurt with flaxseed oil and flour. Total amount of long-chain fatty acids (above C18) in the experimental yoghurt samples were significantly ($P \leq 0.05$) higher compared to control.

Fatty acids like Myristic acid, Palmitic acid, and Palmitoleic acid values were significantly ($P \leq 0.05$) different from control and experimental yoghurt samples. The control yoghurt showed Myristic acid content of 10.30% and experimental yoghurt values are in the range of 5.28–6.35%. The flaxseed oil and flours incorporated yoghurt had palmitic acid in the range of 19.06–21.06% and stearic acid in the range of 7.53–9.53 levels in the extracted fat. In milk fat, saturated fatty acid consist of about 31% palmitic acid, 12% myristic acid, 11% stearic acid and 11% lower (at most 12 carbon atoms) saturated fatty acids and unsaturated fatty acids consist of about 24% oleic acid, 4% palmitoleic acid, 3% linoleic acid (LA) and 1% alpha linolenic acid (ALA). The ratio between omega-6 and omega-3 fatty acids in cow milk fat was 6.01 (National Research Council 1976; Simopoulos 2008).

Moya et al. (1999) studied the thermal oxidation of polyunsaturated fatty acids and reported that a decrease in unsaturation occurred at 150 °C and above. Therefore, linoleic and linolenic acids which are polyunsaturated have high stability at temperature well below 0 °C.

US National Institutes of Health (2000) and International Society for the Study of Fatty acids and Lipids (ISSFL) recommended an ALA dose of 2 g/day. According to ICMR (2010), 1.6 g of ALA is recommended per day for adults. Kolanowski and Weibrodts (2007) reported that fortification of fish oil at 20, 30 and 40 g/kg in 30 g of spreadable fresh cheeses, butter or processed cheeses might provide approximately 180, 270 and 360 mg of omega-3 long chain PUFA. Goh et al. (2006) reported that bioavailability of long chain omega-3 PUFA can be improved by the way of mixing it in a food matrix that accelerates their entry into the mucosal cells. Pszczola (2002) reported that both linoleic and α -linolenic acids are known for their health beneficial effect. Goyal et al. (2016) stated that % ALA content was 1.92 and 10.62 (of total fatty acids) in control and microencapsulated flaxseed oil powder (MFOP) fortified dahi sample, respectively on fresh product. Divya et al. (2013) supplemented buffalo milk with omega-3 fatty acids such as alpha linolenic acid (ALA) and docosahexanoic acid (DHA) and also added partially hydrolyzed guar gum @1% to the milk to meet 25% of daily requirement of dietary fiber.

Conclusion

The sensory evaluation of the experimental samples showed the significantly lowered sensory scores for 2% flax seed flour incorporated yoghurt compared to the control and the experimental sample with 2% flax seed oil and 1% flax seed flour. The TBA values increased with increased incorporation of flaxseed oil and flaxseed flour. A significant difference was found in the content of the polyunsaturated fatty acid C18:3 between control yoghurt and yoghurt with flaxseed oil and flour. Total amount of long-chain fatty acids (above C18) in the experimental yoghurt samples was higher compared with control. The optimized yoghurt which was most accepted at level of 2% flaxseed oil and 1% flaxseed flour contributed 22.80% of ALA accounting for 53% of RDA in 100 g serving size.

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Compliance with ethical standards

Conflict of interest None.

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