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Extraction of Inulin from *Cichorium intybus* and its application as fat replacer in yoghurt

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Abstract

Inulin is significant ingredient used in food industry that functions technologically as a fat replacer often without compromising taste and texture. In this study inulin was extracted from the chicory roots and the effect of inulin addition as a fat replacer on the physiochemical, microbiological and sensory properties of non-fat yoghurt was investigated. The supplementation of chicory inulin reduced the magnitude of firmness in comparison with non-inulin supplemented non-fat yoghurt. Higher values of acidity were observed due to the more microbial fermentation in the inulin containing yoghurt as compared to non-inulin yoghurt and were in the range of 0.56 to 0.75 during storage days. Syneresis in control sample increased from 43.9% to 47.9% during the storage study. However inulin addition at different treatment enhanced syneresis from 44.5% to 47.6%. Inulin addition at various concentrations caused an increase in the TPC due to its probiotic effect. No effects of inuline addition on fat and protein contents were observed. Non-fat yoghurt supplemented with inulin demonstrated sensory behavior better than that of the control yoghurt. The most important effect of the addition of inulin to non-fat yoghurt is an increase in the sensory attributes appearance, body and texture, taste and mouthfeel, overall acceptability. On an average, yoghurt supplemented with 1 to 2% inulin was better in overall acceptance as compared to control yoghurt.

Keywords: Inulin, fat replacer, yoghurt, sensory evaluation, low fat

INTRODUCTION

Recently, the relationship between fat consumption and heart diseases has been accepted and the reduction of dietary animal fat has been recommended by nutritionists (Kucukoner and Haque, [1]. The use of certain plant-derived, non-digestible carbohydrates has potential application for the replacement of fat in foods, maintaining some or all of the desirable technological and sensory-quality attributes of the original full-fat product.

A non-digestible carbohydrate food ingredient that currently has commercial applications as a fat replacer is inulin Franck, [2]. Inulin is a naturally occurring storage polysaccharide present in numerous fruits and vegetables, primarily chicory, Jerusalem artichoke, asparagus and garlic, ranging from 10 to 22% Chiavaro, [3].

The industrial chicory (*Cichorium intybus*) a source of inulin is a biennial plant belonging to the Compositae family. Chicory tuberous roots store inulin with high fructose content (about 94%). Chicory roots contain sugar content of about 70% on dry weight basis almost totally constituted by fructans, with a high mean degree of polymerization (DPm). This fraction is predominantly constituted by inulin. Native chicory inulin is a non-fractionated inulin extracted from fresh roots that always contain glucose, fructose, sucrose and small oligosaccharides Roberfroid, [4]. Inulin is legally classified as food or food ingredients (not as additives). World has recognized the GRAS (Generally Recognized As Safe) status of chicory inulin and can be used without any specific limitations in food products Aryana, [5].

Inulin possesses several functional and nutritional properties that may be used to formulate innovative healthy foods for today's consumer. It is resistant to hydrolysis in both the stomach and small intestine and is classified as dietary fiber ingredients. One of the interesting functions of inulin in human nutrition is related to their prebiotic effect as well as the growth inhibition of pathogens and harmful microorganisms Roberfroid, [6].

The importance of microbiota within the gastrointestinal tract has become evident from studies showing the role of intestinal micro-organisms in the synthesis of fermentation products that provide energy to the colon epithelium. It contributes to the stimulation of the gut immune system, synthesis of vitamin K and resistance to colonization of exogenous bacterial pathogens Hopkins and Macfarlane, [7]. Through positive action on mineral retention leading to increase bone mass achievement and bone mass conservation during ageing. Inulin have reported beneficial effects on bone mineral content (BMC), bone mineral density (BMD) and gastrointestinal absorption of Ca and other minerals Weaver, [8].

Inulin is partly or totally degraded by the intestinal microflora. Fermentation of inulin stimulate the formation of short-chain fatty acids such as acetate, propionate, butyrate and gases, the latter being the preferred energy substrate for colonocytes Kruse, [9]. Furthermore, its use as dietary fibre in man offers other potential health benefits such as reduction in risk of colonic diseases, insulin-independent diabetes, obesity, osteoporosis, cancer blood cholesterol reduction, immune stimulation and enhanced vitamin synthesis Jenkins, [10].

Table 1 Experimental Design for the preparation of low fat yoghurt containing inulin

Samples	Milk fat (%)	Inulin (%)	Total Solids (%)
T_0 (control)	0.1	0	16
T_1 (1% inulin)	0.1	1	16
T_2 (2% inulin)	0.1	2	16
T_3 (3% inulin)	0.1	3	16

The average dietary intake of inulin by humans is estimated to be 1–4 g/d. However, to assure its bifidogenic effect inulin should be added in amount ranging from 3 to 6 g per 100 g or 100 ml and contents of 3-8 g per portion Meyer,[11]. Higher inulin content can modify the texture of dairy products and may significantly influence their sensory quality Tungland and Meyer, [12].

Nutritional and technological characteristics make inulin a very attractive ingredient. This non-digestible carbohydrate is now employed in an increasing number of applications across the food market; dairy and bakery products, beverages, cereals and cereal bars, low-fat spreads, ice cream and confectionary products Meyer, [13]. Inulin exhibits interesting functional properties like: gelation, foams and emulsions stabilizing action. Its use as a natural texture modifier is very interesting, as texture is an important attribute of food products consumer's acceptance. The present study was designed to extract inulin from indigenous source and to study the fat replacing ability of chicory inulin in nonfat yoghurt.

MATERIALS AND METHODS

Extraction of Inulin

Chicory roots were collected manually from the local fields of Faisalabad. Skim milk powder and other raw materials were purchased from the local market of Faisalabad. Inulin was extracted from chicory roots by following the method as described by Passephol, [14]. A lot of two kilograms chicory roots was washed, peeled with the peeling knife and chopped into a fine material in the Blender. The chopped material was boiled in 5 liter distilled water containing sodium metabisulphite solution with a concentration of 100 ppm in a stainless steel kettle, with a vigorous stirring at 95 to 98°C for 10 to 15 min.

After the extraction process the crude extract was filtered through muslin cloth and re-extraction was performed with 3 L distilled water at 95-98°C for a period of 5-7 minute with pressing. Crude chicory root extract was collected with soluble solid contents of 2°B. This extract was further concentrated to 5°B using rotary evaporator. The crude concentrated extract was blended with solution of 5% calcium hydroxide at an operating temperature of 50-60°C for a period of 30 minutes. As a result flocculent formed was precipitated giving a supernatant having a clear bright yellow color. pH of the filtered supernatant increased from 5-6 to10-12. Phosphoric acid (H_3PO_4) of 10% concentration was added to the filtered extract with a vigorous continuous stirring. The excessive coagulate organic material and calcium was precipitated by adjusting the pH to 8-9. The filtrate mixture was then allowed to stay for a period of 2-3 hours at a temperature of 60°C before re-filtering the mixture through Whatman No. 4 filter paper. This clarification process was performed again for the better clarification. Powder charcoal was added to the filtrate with stirring using a glass rod for a period of 15-30 minutes at 60°C to eliminate the coloring substances. The clarified extract was again filtered through Whatman filter paper No.1 and the resultant filtered clear juice was additionally concentrated by rotary evaporator operating at 70°C, to attain concentration of the syrup to a level of 20°B soluble solids. After that it was stored in the ultra low freezer at -60°C until further use.

Cold fractionation and freeze drying of the extract

Syrup with 6-12 °B soluble solid contents was warmed at 65°C and 50 mL sample was stored at 4°C and then -24°C for a period of 24 hours in screw caped centrifuge tubes. Frozen syrup was then subjected to thawing at 25°C. Thawing process caused the inulin precipitation having a dense appearance of white color at the bottom of the screw caped centrifuge tubes. Finally inulin was centrifuged at 2500 g for a period of 10 minutes at 4°C for inulin recovery. Inulin was then subjected to freeze drying process to get a stable inulin product similar to the commercially available inulin powder.

Analyses of inulin

Ash content of inulin was determined gravimetrically using AOAC method 945.46 AOAC 2000, [15]. Total solids of inulin samples were determined gravimetrically following AOAC 2000,[15] method 990.20. The pH of inulin samples were measured with a pH meter (Wellian model-Inolab pH 720, WTW

82362 according to method described by Ong et al., (2007)[16]. A sufficient quantity of representative sample of extract/syrup was taken in a beaker in which electrode of pH meter was immersed and reading was recorded after standardizing the instrument with buffer solutions of pH 4.01 and 7.0.

Preparation of yoghurt

Yoghurt was prepared in the Food Microbiology and Biotechnology Laboratory and Dairy Laboratory, National Institute of Food Science and Technology, University of Agriculture Faisalabad by following the method described by Stelio and Emmanuel, [17]. Inulin was added @ 1%, 2 %, 3% and control with 0%. The prepared yoghurt was stored at a temperature of 4-6°C to check further fermentation and was subjected to physicochemical, microbiological and sensory evaluation.

Physicochemical analysis of yoghurt

The pH of yoghurt samples were measured with a pH meter (Wellian model-Inolab pH 720, WTW 82362) by following the method as described by Ong, [16]. The acidity was determined by direct titration AOAC, 2000, [15]. The fat content of yoghurts were determined by following the method as described by British Standard Institution, [18]. Eleven grams of sample was taken in a butyrometer containing 10 ml H_2SO_4 (specific gravity 1.835) and 1 mL iso-amyl alcohol. Mixed and centrifuged for six minutes at 1100 rpm and noted the reading. Crude protein content was

Table 2: The dilutions were as fallows:

Tube No.	1	2	3	4	5	6	7
Dilution	1:10	1:100	1:1000	1:10000	1:100000	1:1000000	1:10000000
Vol. of yoghurt samples per mL	0.1 (10^{-1})	0.01 (10^{-2})	0.001 (10^{-3})	0.0001 (10^{-4})	0.00001 (10^{-5})	0.000001 (10^{-6})	0.0000001(10^{-7})

determined by Kjeldahl's method as described in Kirk and Sawyer (1991)[19]. Viscosity of the yogurt was determined by means of a Brookfield DV-I viscometer (LVDVE 230) following the method as described by Gassem and Frank, [20]. Firmness of low fat and control yoghurt was performed by following the method as described by Awad, [21]. The Stable Micro Systems texture analyzer (TA-XT plus, Vienna count, surrey GU7 1 YL, UK) was used to analyze the texture of the yoghurt samples. Firmness was estimated from texture analysis graphs. Syneresis of the yoghurt samples at different storage period was determined by following the method as described by Rodarte, [22]. Yoghurt samples (30 g) were centrifuged at 222 g for 10 minutes at 4°C. After centrifugation, the supernatant was poured off, weighed and recorded as percentage synersis.

Microbiological analysis of yoghurt

Total plate count of bacteria was estimated by making serial dilutions for each sample by the method as recommended by Cappuccino and Sherman, [23].

Sensory evaluation

For assessment of overall acceptability of inulin enriched yoghurt was done by a panel of 5 judges among the faculty and research scholars at NIFSAT, University of Agriculture, Faisalabad. Panel constituted judges who were trained and familiar for yogurt's attributes and showed their willingness. Appearance, body and texture, taste and mouthfeel and over all acceptability were rated on a 5 point scale scoring 5 for excellent, 1 for poor. As recommended by IDF, [24]. Taste and mouthfeel were given priority over the others, so its scores were multiplied by 2. Total scores were obtained by adding the scores of all attributes. Yoghurt samples were coded with numbers and presented together to panel members in day light. Water was provided for rinsing mouth after each sample.

Statistical analysis

Data obtained was subjected to statistical analysis using two factorial Completely Randomized Designs (CRD) and ANOVA techniques as described by Steel, [25] to evaluate the quality and acceptability of yoghurt supplemented with inulin.

Results and Discussion

The present project was designed to extract inulin from indigenous source like chicory roots and to investigate the effect of Inulin addition as a fat replacer on the physicochemical, microbiological and sensory attributes of fat free yogurt. Inulin was extracted from chicory roots. Yoghurt was prepared by reconstituting the skim milk powder. Prior to pasteurization inulin was added at different concentrations 0, 1, 2 and 3%. Pasteurized milk was inoculated with starter culture at 42°C and stored in refrigerated condition at 4°C after incubation. Physicochemical, microbiological and sensorial properties of the product were studied during shelf life of 0, 7th, 15th days interval.

Composition of inulin powder and reconstituted skimmed milk

The results regarding the analysis of inulin powder are listed in Table (2). The powder contained 94.6% (w/w) solids and 5.4 % ash. It appeared as an off-white powder. No sweetness (compared to sucrose=100) of inulin powder was observed. Solubility in water at 25°C (g/l) was found to be 95 of inulin powder. These results are according to the findings of the Paseephol, [26], who recovered inulin from the Jerusalem artichoke tubers using the same extraction conditions with a composition of 97% total solid contents, 77% inulin and ash contents of 6.1%.

The solubility of inulin in water is temperature dependant, varying from 6% at 10°C to 35% at 90°C. Low solubility at low temperatures is a useful property which can be employed to separate high molecular weight inulin fractions from aqueous solutions. As the temperature decreases, the heavier MW inulin tends to settle at the bottom of the container and push the low-MW inulin and mono and disaccharides upward Moerman, [27].

Reconstituted skim milk was analyzed for different parameters like pH, acidity, fat, SNF, protein and total solids. Reconstituted skim milk contains 0.1% fat, 5.2% protein, 10.7% SNF, total solids 16% having a pH of 6.6 and titratable acidity 0.11%. The concluded results are given below in Table (3).

Yoghurt analysis

Yoghurt was subjected to physicochemical (pH, acidity, fat, protein, syneresis, hardness and viscosity), microbiological (Total plate count: TPC) and sensory analysis (body & texture, color & appearance, taste & mouth feel and overall acceptability). The evaluation was done at 0, 7th and 15th days of shelf life storage.

Physicochemical analysis of yoghurt

pH

The results indicated that pH of the yoghurt differed significantly by the difference in the treatments and storage time. However, non-significant interaction was observed between treatments and the storage days.

Table 3. Analysis of inulin powdered extract

Composition	Crude Extract
Total solids %	94.6 ± 1.33%
Ash %	5.4 ± 0.12%
Color	Off-white
pH	6.5 ± 0.62
Taste	Neutral
Sweetness (compared to sucrose=100)	None
Solubility in water at 25°C (g/l)	95 ± 0.34

Analysis were performed in duplicate
Values are given as mean + SD

The results given in Table 4 indicated that pH of yoghurt ranged from 4.7 to 3.9 between the treatments during different storage time. The storage time showed a significant impact on pH exhibiting a decreasing trend. However, pH decreased from 4.77 to 4.51 in T_0. Similarly pH decreased from 4.70 to 4.45, 4.70 to 4.43 and 4.65 to 4.39 in T_1, T_2 and T_3 respectively of yoghurt samples treated with different concentrations of inulin. At 0 day the highest pH value (4.77) was observed in yoghurt sample T_0 and lowest (4.65) was exhibited in the sample T_3. After 7 and 15 days there was remarkable change in pH of yoghurt supplemented with different concentrations of inulin.

These results are according to the findings of Maha, [28] who reported that there is no significant effect of inulin stabilizer on pH value while Kamaruzzaman, [29] reported that the pH decreased during the storage of yoghurt due to the production of lactic acid.

Acidity

The results regarding acidity of yoghurt indicated that acidity of the yoghurt differed significantly (P<0.01) by the difference in the treatments and storage days. However, non significant interaction was observed between treatments and the storage days.

The results given in Table 4. Showed that acidity of yoghurt containing different concentrations of inulin ranged from 0.35 to 0.94% between the treatments during different storage time. The storage days showed a highly significant impact on acidity exhibiting an increasing trend. However, acidity increased from 0.35 to 0.57% in T_0. Similarly acidity increased from 0.57 to 0.71%, 0.62 to 0.78% and 0.72 to 0.94% in T_1, T_2 and T_3 respectively of yoghurt samples treated with different concentrations of inulin. On 0 day analysis highest acidity (0.72%) was recorded in the sample T_3 while lowest acidity (0.35%) was noted in T_0. After 7 and 15 days acidity of yoghurt samples supplemented with different concentrations of inulin also increased.

These findings are in accordance with the findings of Susan, [30] who stated that titratable acidity of the samples was not affected by different ratios of inulin and increases were found to be significant during storage (P < 0.05). Some differences were observed during storage and titratable acidity was highest at day 15. Khalifa, [31] stated that the acidity increases with prolonged storage time. Chougrani, [32] and Salvador and Fiszman, [33] also concluded that the acidity of yoghurt increases with the increased storage period due to microbial activity and lactose conversion into lactic acid.

Syneresis

The syneresis of the yoghurt differed significantly (P<0.01) by the difference in the treatments and storage days as well as interaction between treatments and the storage days.

The results given in Table 6 specified that syneresis of yoghurt containing different concentrations of inulin ranged from 48.84 to 43% between the treatments during different storage time. The storage days showed a highly significant impact on syneresis exhibiting an increasing trend. However, syneresis increased from 43.00 to 46.4% in T_0. Similarly syneresis increased from 43.26 to 46.76%, 43.93 to 48.61% and 45.47 to 49.91% in T_1, T_2 and T_3 respectively of yoghurt samples treated with different concentrations of inulin. On 0 day analysis highest syneresis (45.47%) was recorded in the sample T_3 while lowest syneresis (43%) was noted in T_0. After 7 and 15 days syneresis of yoghurt samples supplemented with different concentrations of inulin also increased.

These results are in line with the findings of Isleten and Karagul, [34] reported that syneresis of yoghurt increased with the increased storage period. Folkenberg, [35] reported a negative correlation between syneresis and firmness in inulin containing yogurts; they observed that syneresis was more pronounced in inulin containing yogurts.

Fat

The results showed that fat content of the yoghurt differed significantly (P<0.01) by the difference in the treatments and storage time. However, non-significant interaction was observed between treatments and the storage time.

It is obvious from the results given in Table 5 that fat content of yoghurt containing different concentrations of inulin ranged from 0.06 to 0.15% between the treatments during different storage time. The storage days showed a highly significant impact on fat content of yoghurt exhibiting a decreasing trend. However, fat % decreased from 0.15 to 0.10% in T_0. Similarly fat decreased from 0.13 to 0.08%, 0.12 to 0.08% and 0.11 to 0.06% in T_1, T_2 and T_3 respectively of yoghurt samples treated with different concentrations of inulin. On 0 day analysis highest fat content (0.15%) was recorded in the sample T_0 while lowest fat (0.11%) was noted in T_3. After 7 and 15 days fat percentage of yoghurt samples supplemented with different concentrations of inulin also decreased.

These results agreed with the findings of Khan, [36] who reported that fat content decreased during storage of cultured milk. Reduction in fat content of yoghurt samples under the influence of storage appeared due to lipolytic activity of micro flora or due to acidic pH during the storage for over a long period of time. However, no rancidity was detected because of low storage temperature.

Viscosity

The viscosity of the yoghurt also differed significantly (P<0.01) by the difference in the treatments and storage time. However, non-significant interaction was observed between treatments and the storage time.

The results given in Table 6 indicated that viscosity of yoghurt supplemented with different concentrations of inulin ranged from 3310.93 to 5504.00 cP between the treatments during different storage time. The storage time showed highly significant impact on viscosity exhibiting a decreasing trend. However, viscosity decreased from 4539.00 to 3310.93 cP in T_0. Similarly viscosity decreased from 5504.00 to 3530.20 cP, 4848.05 to 3459.33 cP and 4876.27 to 3480 cP in T_1, T_2 & T_3 respectively of yoghurt samples treated with different concentrations of inulin. The highest value (5504.00 cP) of viscosity was recorded in yoghurt sample T_1 while lowest (4539.00 cP) was observed in sample T_0 at 0 day storage. After 7 and 15 days viscosity of yoghurt samples supplemented with different concentrations of inulin also decreased.

The results of the present study are in accordance with Gassem and Frank, [37] they reported that viscosity of yoghurt was decreased with the storage time. Similar results of decrease in viscosity over storage time were reported by Farooq, [38] for plain yoghurt.

Gel Strength/Firmness

The firmness of the yoghurt differed significantly (P<0.01) by the difference in the treatments and storage time as well as interaction between treatments and storage time.

The results given in Table 6 cleared that gel strength/ firmness of yoghurt ranged from 17.24 to 285 N between the treatments during different storage time. The storage time showed highly significant impact on firmness exhibiting an increasing trend. However, firmness increased from 247.21 to 285 N in $T_{0.}$ Similarly firmness increased from 202.23 to 237 N, 166.92 to 220 N and 147.24 to 216 N in T_1, T_2 and T_3 respectively of yoghurt samples treated with different concentrations of inulin. The highest value (247.21) of firmness was recorded in yoghurt sample T_0 while lowest (147.24) was observed in sample T_3 at 0 day storage. Increase in the firmness after 7, 15 days of storage and fluctuations were observed within the treatments.

Salvador and Fiszman, [33] indicated results which are in line with present study. Whereas Uprit and Mishra, [39] studied the textural properties of press chilled acid coagulated curd (Paneer) and reported that decrease in hardness can be explained by an increase in moisture content, they correlated the decrease in hardness of the curd to the decrease in fat content and attributed it to the less compact protein matrix.

Protein

The results showed that protein content of the yoghurt differed significantly (P<0.01) by the difference in the treatments and storage time. However, non-significant interaction was observed between treatments and the storage days.

The results (Table 5) indicated that protein content of yoghurt supplemented with different concentrations of inulin ranged from 4.92 to 4.54% between the treatments during different storage days. The storage days showed non-significant impact on protein exhibiting no effect. However, protein ranged from 4.92 to 4.85% in $T_{0.}$ Similarly protein content ranged from 4.84 to 4.90%, 4.66 to 4.74% and 4.54 to 4.65 in T_1, T_2 & T_3 respectively of yoghurt samples treated with different concentrations of inulin. The highest value (4.92%) of protein was recorded in yoghurt sample T_0 while lowest (4.54%) was observed in sample T_3 at 0 day storage. No significant effect on protein % of yoghurt samples were observed after 7, 15 days of storage and fluctuations were observed within the treatments.

The results obtained agree well with the finding of Maha, [28] and Tamime and Robinson, [40] who stated that there is difference in the protein values during storage period but contradicts regarding the treatments (inulin addition effect). The semisolid texture of set yogurt gel is a result of the development of a three dimensional network of milk proteins. The main factor responsible for milk gelation is a reduction in high net negative charge on the casein micelles because of the liberation of acids from microbial activity. During fermentation, casein micelles, together with denatured whey proteins, aggregate into chains and clusters through hydrophobic and electrostatic bonds, governing the structure of yogurt. Aggregation of casein micelles starts at a pH of ~5.3. Further pH reduction to below 5.0 causes a more complex and extensive interconnection of casein micelles and the gel attains its maximum firmness at pH 4.6, the isoelectric point of casein Tamime, [41].

Microbial Analysis of yoghurt

Total Plate Count

The total plate count of the yoghurt differed significantly (P<0.01) by the difference in the treatments and storage time. However, non-significant interaction was observed between treatments and the storage days.

Table 4. Chemical composition of reconstituted skim milk used in the manufacture of experimental yogurt samples

Parameter	Values
pH	6.6 $\pm$ 0.25
Acidity %	0.11 $\pm$0.01
Fat %	0.1 $\pm$0.001
Protein%	5.2 $\pm$0.02
SNF %	10.7 $\pm$0.02
Total solids %	16 $\pm$ 0.21

Analysis were performed in duplicate values are given as mean + SD

The results given in Table 7 showed that total plate count of yoghurt supplemented with different concentrations of inulin ranged from 7.10 x106 to 9.34 x106 between the treatmens time. The storage time showed significant impact on total plate count of yoghurt exhibiting an increasing trend. However, total plate count of yoghurt ranged from 7.10 x10^6 to 8.41 x10^6 in $T_{0.}$ Similarly total plate count ranged from 7.23 x10^6 to 8.68 x10^6, 7.63 x10^6 to 8.8 x10^6 and 7.71 x10^6 to 9.34 x10^6 in T_1, T_2 & T_3 respectively of yoghurt samples treated with different concentrations of inulin. The highest value (7.71 x10^6) of total plate count was recorded in yoghurt sample T_3 while lowest (7.10 x10^6) was observed in sample T_1 at 0 day storage. After 7 and 15 days viscosity of yoghurt samples increased with different concentrations of inulin also increased. However fluctuations were observed within the treatments. The reason to this increase in the total plate count of yoghurt is that inulin acts as a prebiotic substance.

Table 5. Effect of inulin on pH and acidity of yoghurt

Parameter	Samples	Storage Days		
		0	7	15
pH	T_o	4.77	4.94	4.51
	T_1	4.70	4.77	4.45
	T_2	4.70	4.65	4.43
	T_3	4.65	4.50	4.39
Acidity %	T_o	0.35	0.42	0.57
	T_1	0.57	0.64	0.71
	T_2	0.62	0.72	0.87
	T_3	0.72	0.82	0.94

Table 6. Effect of inulin on protein and fat contents of yoghurt

Parameter	Samples	Storage Days 0	7	15
Protein%	T_1	4.92	4.85	4.91
	T_2	4.84	4.90	4.86
	T_3	4.66	4.74	4.67
	T_3	4.54	4.50	4.65
Fat%	T_1	0.15	0.12	0.10
	T_2	0.13	0.10	0.08
	T_3	0.12	0.10	0.08
	T_3	0.11	0.09	0.06

Table 7. Effect of inulin on viscosity (cP) and syneresis of yoghurt

Parameter	Samples	Storage Days 0	7	15
Viscosity (cP)	T_o	4539.00	4393.50	3310.93
	T_1	5504.00	5383.43	3530.20
	T_2	4848.00	4489.76	3459.33
	T_3	4876.27	4634.63	3480.70
Syneresis (%)	T_o	43.00	44.18	46.40
	T_1	43.26	44.66	46.76
	T_2	43.93	45.78	48.61
	T_3	45.57	47.51	49.91
Gel strength/firmness (N)	T_o	247.21	255.70	285.17
	T_1	202.23	214.18	237.40
	T_2	166.92	208.00	220.25
	T_3	147.24	205.00	216.55

Table 8. Effect of inulin on microbial analysis (total plate count) yoghurt

Parameter	Samples	Storage Days 0	7	15
TPC (cfu/ml)	T_o	7.10×10^6	8.406×10^6	7.61×10^6
	T_1	7.23×10^6	8.68×10^6	7.78×10^6
	T_2	7.63×10^6	8.8×10^6	7.88×10^6
	T_3	7.71×10^6	9.34×10^6	8.11×10^6

All the results were in agreement with the Aryana, [5] who showed a significant effect during storage interval and inulin addition on the TPC of yoghurt. Similarly Donkor, [42] observed that chicory-based inulins were favoured carbon source for probiotic bacteria, hence increasing the growth performance and sustaining the viability during storage.

Sensory evaluation of yoghurt

All the samples of prepared yoghurt were sensorily evaluated with storage intervals of 0, 7 and 15 days. Five judges were provided with printed Performa as shown in chapter 3. The sensory evaluation data for example body and texture, taste and mouthfeel, color and appearance, and overall acceptability were then processed for statistical analysis. These four fundamental parameters associated with the quality and acceptability of dairy product and yoghurt as affected by storage is discussed below.

Taste and Mouthfeel

Different treatments and storage days showed highly significant effect on the scores assigned to taste and mouthfeel of yoghurt samples as well as the interaction between treatments and storage days also showed significant effect.

The results presented in Table 8 indicated that the scores assigned to taste and mouthfeel of yoghurt supplemented with different concentrations of inulin ranged from 8.31 to 6.5 between treatments during different storage days. However, taste and mouthfeel score varied from 7.78 to 7.11 in T_0. Similarly taste and mouthfeel scores decreased from 8.25 to 7.44, 8.31 to 7.12 and 7.48 to 6.5 in T_1, T_2 and T_3 respectively of yoghurt samples treated with different concentrations of inulin. Highest value (8.31) was recorded in T_2 and lowest (6.5) in T_3 at 0 day. Results indicated that there was decrease in scores assigned to taste and mouthfeel of yoghurt prepared from different concentrations of inulin during 7 and 15 day of storage time.

Taken together it appears that inulin contributes to an

Table 9. Effect of inulin on sensorial characteristics of yoghurt

Parameter	Samples	Storage Days 0	7	15
Taste and Mouthfeel	T_o	7.78	7.23	7.11
	T_1	8.25	4.54	7.44
	T_2	8.31	7.41	7.12
	T_3	7.482	6.94	6.5
Body and texture	T_o	4.29	3.91	3.52
	T_1	4.25	3.61	3.73
	T_2	4.58	4.07	3.39
	T_3	4.42	3.50	3.00
Color and Appearance	T_o	4.29	3.68	3.4
	T_1	4.45	4.33	3.71
	T_2	4.2	4.41	4.07
	T_3	4.37	4.37	4.25
Overall Acceptability	T_o	4.2	3.81	3.45
	T_1	4.55	4	4.21
	T_2	4.37	3.65	3.11
	T_3	4.33	3.4	3

improved creamy mouthfeel by enhancing the attribute like airy and having a positive effect on thickness and stickiness. The overall creamy mouthfeel scores of the yoghurt samples were not significantly different, but a clear trend was noted for control yoghurt and 3% inulin in creamy mouthfeel.

Results show that effect of storage was highly significant on taste and mouthfeel of all treatments Kip, [43] also reported a decrease in taste and mouthfeel of yoghurt during storage intervals. The addition of inulin did not have a bad impact on mouthfeel rather it was quite acceptable because of its long chain, has a fat-like mouthfeel which was encountered in the fat-free yogurts Niness, [44] reported that inulin has been successfully used to replace fat in yoghurt, baked goods, table spreads, fillings, dressings and frozen desserts. These results are in favor of Kip, [45] and Weenen, [49] who found that an optimum is seen for creamy mouthfeel at an inulin concentration between 1.5% and 4%.in acidity at 4^{o}C.

Body and texture

The body and texture of the yoghurt differed significantly (P<0.01) by the difference in the treatments and storage time as well as interaction between treatments and storage time.

The results presented in Table 8 indicated that the scores assigned to body and texture of yoghurt supplemented with different concentrations of inulin ranged from 4.58 to 3 between treatments during different storage days. However, body and texture score varied from 4.29 to 3.52 in T_0. Similarly body and texture scores decreased from 4.25 to 3.75, 4.5 to 3.4 and 4.4 to 3.0 in T_1, T_2 and T_3 respectively of yoghurt samples treated with different concentrations of inulin. Highest value (4.5) was recorded in T_2 and lowest (4.29) in T_0 at 0 day. Results indicated that there was decrease in scores assigned to body and texture of yoghurt prepared from different concentrations of inulin during 7 and 15 day of storage days.

Body and texture of the yoghurt is influence by many factors including acidity and total solids. This was due to less acidity and as well as due to low proteolytic activity in yoghurt samples with less protein contents other than the plain yoghurt and increase in total solid contents could be the second reason for higher body and texture score of yoghurt. The results are in line with the findings of Kucukoner, [1] they reported a decrease in body and texture of yoghurts during storage.

Texture was affected significantly during storage in all experimental yoghurts; however addition of inulin also acts as stabilizers and had a remarkable improvement in scores of experimental yoghurts for body and texture, which is in accordance with findings of Kip, [43].

Color and Appearance

The results showed that color and appearance of the yoghurt differed significantly (P<0.01) by the difference in the treatments and storage time as well as their interaction.

Highest value (4.5) was recorded in T_1 and lowest (4.25) in T_3 at 0 day. Results indicated that there

The results are in agreement with the findings of Kayanush and McGrew, [48]. who reported a decrease in score of color and appearance of yoghurts during inulin addition and storage period.

Overall Acceptability

Different concentrations of inulin and storage days showed highly significant effect on the scores assigned to overall acceptability of yoghurt. However the interaction of treatments and storage days and time showed non-significant effect on the scores assigned to the overall acceptability of yoghurt

The results (Table 8) showed that the scores assigned to overall acceptability of yoghurt supplemented with different concentrations of inulin ranged from 5.5 to 3 for different storage time periods. However, overall acceptability scores varied from 4.2 to 3.4 in T_0. Similarly overall acceptability scores decreased from 4.5 to 4.0, 4.4 to 3.1 and 4.3 to 3.0 in T_1, T_2 and T_3 respectively of yoghurt samples treated with different concentrations of inulin. Highest value (4.5) was recorded in T_1 and lowest (4.3) in T_3 at 0 day. Results indicated that there was decrease in scores assigned to overall acceptability of yoghurt prepared from different concentrations of inulin. There was a decreasing trend in the overall acceptability with the increase in storage time of all the yoghurt samples.

Weenan, [49] reported similar results and found that there is little bit change in the overall acceptability of the product during storage. Di-Criscio, [45] reported yoghurt with 2% inulin did not alter physical or sensory characteristics significantly; however yoghurt with high inulin concentration altered the physical and sensory characteristics. Our results support yoghurt with up to 2% inulin is acceptable.

The preliminary study of this project revealed that chicory grown in our country would be useful for the production of inulin as its tubers contain 13% total fructans on fresh basis which is almost 85% of the total carbohydrates. The most important effect of the addition of inulin to non-fat yoghurt is an increase in the sensory attributes appearance, body and texture, taste and mouthfeel, overall acceptability. These attributes positively affect creamy mouthfeel in low-fat yoghurts. On average yoghurt supplemented with 1 to 2% inulin was better in overall acceptance as compared to control yoghurt. It was concluded that the increased use of inulin in fat free yoghurt negatively influenced some physical parameters and organoleptic scores. However, yoghurt containing 1% of inulin showed better characteristics to the control yoghurt. For the manufacturing of low fat yoghurt, inulin should be added at a level of 1%, higher values were not satisfactory.

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