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Impact of Dietary Fiber (Inulin and Resistant Starch) on the Quality Parameters of Low Fat Cheddar Cheese from Buffalo Milk

Mian Shamas Murtaza[1,*], Aysha Sameen[2], Saima Rafique[3], Muhammad Shahbaz[1,*], Nabeela Gulzar[4], Mian Anjum Murtaza[5], Umar Farooq[1] and Iram Hafiz[6]

[1]Department of Food Science and Technology, MNS-University of Agriculture, Multan
[2]National Institute of Food Science and Technology, University of Agriculture, Faisalabad-38040
[3]Department of Food Science and Technology, Faculty of Agriculture, University of Poonch, Rawalakot, Azad Jammu and Kashmir, Pakistan
[4]Department of Dairy Technology, University of Veterinary and Animal Sciences, Lahore
[5]Institute of Food Science and Nutrition, University of Sargodha, Sargodha 40100
[6]Institute of Chemistry, University of Sargodha, Sargodha 40100

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MSM and AS presented the idea. AS helped in graphics. MSM conducted the research. MSM, MS and IH wrote the manuscript. SR, and MAM reviewed the manuscript.NG and IH performed statistical analysis. UF validated the results.

Key words
Melt-ability, Flow-ability, Gumminess, Cohesiveness, Hardness, Physicochemical characteristics of cheese, Functional characteristics of cheese, Textural profile of cheese, Low fat cheese.

ABSTRACT

Low fat cheese is the demand of this era due to overconsumption and sedentary life style and increase in diseases like hypertension, obesity, cardiovascular diseases *etc*. Inulin is a food ingredient that belongs to a class of carbohydrates known as fructans with degree of polymerization range from 2-70. Resistant starch is obtained by chemical and enzymatic treatment of starch to increase its properties. These have functional and health-promoting properties as they reduce caloric value, add dietary fiber and endorse prebiotic effects. These are frequently used in industrially processed dairy as a bulking agent for fat replacement, textural modifications and organoleptic improvements. Hence, these can be fruitfully be used in manufacturing different kind of cheeses to have reduced or low fat, texturized or symbiotic product. Present study designed to evaluate the effect of different levels of inulin and resistant starch in low fat cheddar cheese to improve its quality. Different levels have significant effect on the physic-chemical (moisture, fat, protein, ash,) characters. Melt-ability and flow-ability showed inverse relationship with levels of inulin. Melt-ability and flow-ability decreased by increasing level and increasing hardness. Yield calculation showed non-significant effect within levels but significant effect as compared with control. The fat substituting property is based on its ability to stabilize the structure of aqueous phase which creates an improved creaminess. The addition of inulin and resistant starch as fat replacement improved the sensory characteristics of low fat cheese samples when added up to the level of 0.5% and 1.0%, respectively.

INTRODUCTION

Milk is a complete diet. Pakistan has large milk production with 59.75 million tonnes per year (GoP, 2019). There are more than 5000 cheese varieties exist in the world that can be classified on the basis of flavor, color, ripening, moisture, starter culture, manufacturing procedure *etc*. Cheese manufacturing is the oldest way to preserve milk by condensing milk to many folds (Varnam and Sutherland, 2009). Cheese is the concentrated form of milk solids mainly milk protein and fat. There is a networking of protein and fat in cheese that makes its structure (texture) (Omotosho *et al*., 2011). Cheddar cheese has hard texture with more solids and less moisture and have more shelf life as compared to other cheese varieties. Despite of importance of fat in cheese manufacturing and texture development it also causes many problems in the human body due to excess consumption of saturated fatty acids like cardiovascular problems, high cholesterol level, arthritis, memory loss, weight gain and obesity. In Pakistan, these chronic diseases are the cause of 42% deaths (GoP, 2016). Consumers have certain health concerns owing to changing life style, like less fat, fewer calories, less cholesterol or less sugar (Verbeke, 2006). The demand of low fat cheese is increasing day by day. Reduced and low fat cheeses are desired based on composition but

* Corresponding authors: shahbazft1658@gmail.com; shamas.murtaza@mnsuam.edu.pk
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often fell short in overall quality (Murtaza, 2016). Some of the major problems with fat reduction in cheese are the development of a firm texture that does not break down during mastication, weak gel network between protein and fat, bland flavor and taste and yield reduction, which are undesirable (Correll, 2011). Fat replacers are ingredients intended to be used as alternate of natural fats with the objective of reduction in the caloric value.

Inulin is a food ingredient that belongs to a class of carbohydrates known as fructans with degree of polymerization range from 2-70. Chemical structure is linear fructan with (2-1)-linked β-D fructosyl units. Fructan with degree of polymerization less than 10 are called fructo-oligo-saccharides (Shakeel *et al*., 2003). It has functional and health-promoting properties as they reduce caloric value, add dietary fiber and endorse prebiotic effects. Inulin is frequently used in industrially processed dairy and non-dairy products as a bulking agent for fat replacement, textural modifications and organoleptic improvements. Hence, inulin can fruitfully be used in manufacturing different kind of cheeses to have reduce or low fat, texturized and/or symbiotic product (Karimi *et al*., 2015). Koca and Metin (2004) considered the possibility of obtaining low-fat fresh kashar cheese with a 70% fat reduction using long-chain inulin. They reported that their low-fat control cheese, due to its high protein content, was significantly harder, more elastic, gummier and chewier than the full-fat control cheese. Fadaei *et al*. (2012) also reported that inulin has an excellent water binding capacity which inhibits syneresis in spreads and fresh cheeses.

Starches incorporate multiple functional characteristics through processes like cross-linking of protein, hydrolysis of acid and substitutions. These processes can be used to perform many functions within a food matrix (BeMiller and Whistler, 1996). Starches of different plant origins can be used in production of low fat or imitation cheeses as fat substitute, since the interaction and networking of protein and starch system plays quite significant role in improving macroscopic properties of cheeses such as flow-ability, mouth-feel, stability and texture (Noronha *et al*., 2007). Natural and modified starches are being used in low fat cheese to improve the functional and textural properties (Mounsey and Riordan, 2008).

Resistant starch is the combination of common starch and the products obtained by degradation of starch which are not absorbed in the small intestine (Asp and Bjorck, 1992). In this research work Type 4 of resistant starch is used which is chemical and enzymatically modified. In Type 4 resistant starch functional properties are changed to increase the proportion of resistant starch in comparison with starch. Type 4 resistant starch is obtained by the reaction of starch with bi-or polyfunctional reagents, resulting in resistance to enzyme attack. Phosphated di-starch, a modified RS made from high-amylose maize starch, is currently used as a food additive (Sajilata *et al*., 2006). Resistant starch is of particular interest to food processors and nutritionists because it has the potential to exert physiological benefits and produce better quality products. The prominent functional features of resistant starch are the low water-holding capacity, stability towards high shear and temperature (Abbas *et al*., 2010). These starches can be used as fat replacer or mimetic for nutritional value and correction of textural defects in low fat cheeses.

MATERIALS AND METHODS

Raw material procurement

Fresh Buffalo milk was obtained from dairy farm. Dietary fiber (inulin and resistant starch Type 4), lypholized mesophilic starter culture (*Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*) and rennet (double strength Chy-max, 500000 MCU/mL, Pfizer Inc., Milwaukee, WI, USA) were purchased from local market.

Cheddar cheese preparation

Normally milk is standardized at 4% fat level for cheddar cheese. For manufacturing of low fat cheese milk was standardized at 2% fat level with the addition of dietary fiber at different concentrations shown in Table I following the procedure as described by Murtaza (2016).

Table I.- Treatment plan.

Treatments	Fat (%) in milk	Inulin (%)	Resistant starch (%)
Control (C+)	4	-	-
Neg. control (C-)	2	-	-
I_1	2	0.5	
I_2	2	1.0	
I_3	2	1.5	
RS_1	2		0.5
RS_2	2		1.0
RS_3	2		1.5

I, insulin; RS, resistant starch

Quality parameters like composition (physico-chemical) functional characteristics (melt-ability, flow-ability) textural characteristics (hardness, cohesiveness, springiness, gumminess, chewiness) and sensory properties were analyzed after 30 days of cheese sample ripening in triplicates (n=3).

Physico-chemical analysis

The cheese samples were analyzed for moisture by oven drying (AOAC, 2000), pH using pH meter (Ong *et* *al*., 2007), acidity by titration method (AOAC, 2000), protein by Kjeldhal method (IDF, 2006), fat by Gerber method (Marshal, 1993) and ash content by igniting the cheese samples (AOAC, 2000).

Analysis of functional characteristics

Melt-ability was determined according to Zisu and Shah (2007). Flow-ability of cheese samples was determined according to Guinee *et al*. (2002). Cheese yield was calculated as the percent of the weight of finished cheese attained divided by weight of the milk used for this trial (Sipahioglu *et al*., 1999).

Textural profile analysis

Texture profile of each sample was assessed by performing the texture profile analysis (TPA) on TA-XT Plus Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK) using compression plate according to Zisu and Shah (2007).

Sensory evaluation

Low fat Cheddar cheese samples were evaluated by a panel of judges for sensory perception on a hedonic rating scale (0–9) to assess the impact of fat reduction and addition of dietary fiber as described by Awad *et al*. (2004).

Statistical analysis

Duncan's multiple range test using Statistica 8.1 using completely randomized design was used to assess the significance of work (Montgomery, 2013).

RESULTS AND DISCUSSION

Physico-chemical analysis

Addition of dietary fiber in the form of inulin and resistant starch has significant effect ($P < 0.05$) on the different physic chemical parameters like moisture, fat, protein, ash, pH and acidity *etc*. (Table II). Moisture is the major component of cheese which acts as plasticizer in the protein matrix, thereby making it less elastic and more susceptible to fracture upon compression (McSweeney and Souse, 2000). The highest moisture content (47.50%) was recorded in cheese sample RS_1 while the lowest moisture content (38.40%) was observed in positive control cheese sample C+. Table II shows that moisture content of all cheese samples varied significantly due to various concentrations of dietary fiber used in preparation of low fat cheese and from the above discussion we can conclude that cheese samples having dietary fiber and low fat retain more moisture. It means the inulin and resistant starch had water binding capacity. Fat is responsible for characteristic texture, flavor and aroma of cheese. The fat in Cheddar cheese plays a key role in overall acceptance and effect cheese body and texture by filling interstitial spaces in the protein and mineral matrix (Kucukoner and Haque, 2006). Lowest fat (16.14%) was recorded in negative control C- and all other treatments having dietary fiber have more fat than the negative control due to the moisture and fat binding property of fiber (Esmaeil *et al*., 2010). Protein especially casein form a three dimensional network and hold the Ca ions that play significant role in the firmness of the end product (Murtaza *et al*., 2017; Guinee *et al*., 2004). Cheese is a concentrated protein matrix with entrapped fat and moisture; therefore, the amount of protein in the matrix increases as the fat level decreases (Van *et al*., 2013). These results were expected and confirmed the work of other investigators like Brummel and Lee (1990). All the treatments having more proteins those have less fat. Addition of dietary fiber did not significantly affect ash content because dietary fiber did not provide minerals and during charring it completely burnt, that is confirmed by the investigation of Sengul *et al*. (2006) according to which addition of dietary fiber had no direct contribution to ash contents. pH is an important attribute that effect almost all facts of cheese quality including flavor, texture and appearance (Sattar *et al*., 2018; Pastorino *et al*., 2003).

Table II.- Physico-chemical composition of full fat and low fat cheddar cheese.

Treatments	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	pH	Acidity (%)
C+	38.40±1.02d	27.43±0.71d	32.30±0.68a	3.42±0.17a	5.20±0.04c	0.91±0.02a
C-	42.81±1.10c	30.62±0.60bc	16.14±0.42d	3.44±0.19a	5.27±0.08b	0.86±0.04b
I_1	46.3±0.27ab	30.68±0.58b	16.32±0.36c	3.37±0.19a	5.31±0.03ab	0.83±0.02b
I_2	44.9±0.18b	31.12±0.64c	16.51±0.38bc	3.38±0.14a	5.37±0.04a	0.78±0.02c
I_3	44.0±0.93bc	31.60±0.67cd	16.99±0.24b	3.41±0.15a	5.39±0.07a	0.77±0.03c
RS_1	47.5±1.03a	31.18±0.54a	16.99±0.40bc	3.37±0.18a	5.33±0.02ab	0.84±0.03b
RS_2	45.4±0.92b	32.52±0.54bc	16.77±0.41bc	3.41±0.20a	5.33±0.05ab	0.83±0.03bc
RS_3	44.4±0.96bc	32.09±0.67c	17.06±0.23b	3.38±0.14a	5.38±0.07a	0.79±0.02bc

Values sharing same letters in one column are statistically non-significant from each other while values sharing different letters in one column are statistically significant from each other. For details of treatments, see Table I.

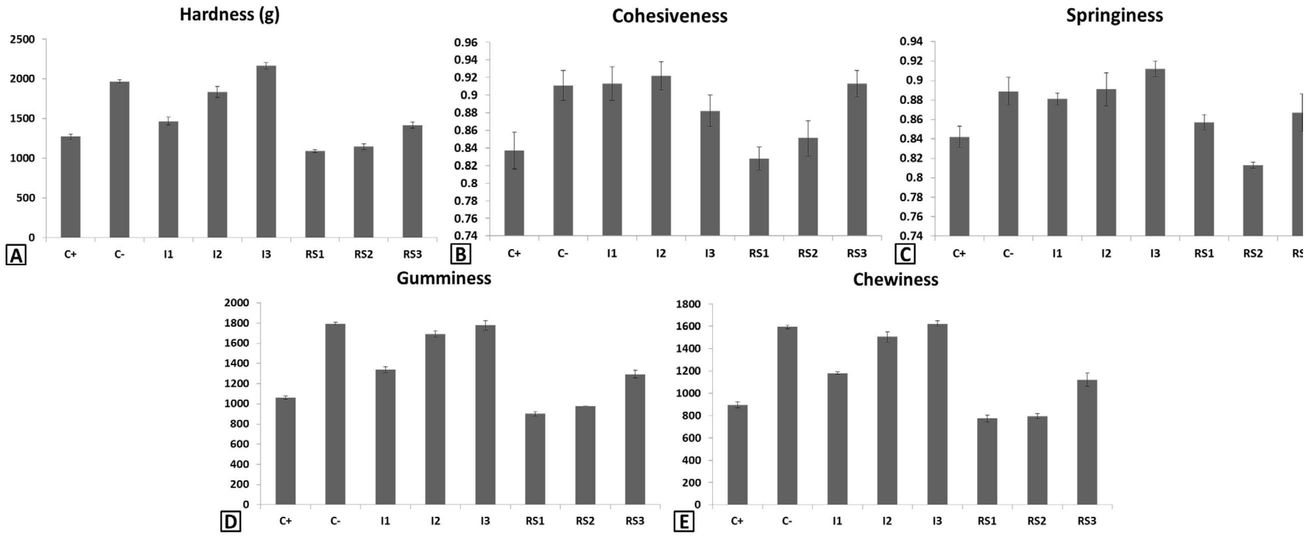

Fig. 1. Texture profile of low fat cheddar cheese.

The emulsification, peptization and ability of casein particles to interact in a heat induced gel structure were largely dependent on physiochemical state of the protein, which depend on pH and ionic composition and strength (Guinee *et al*., 2004). Lowest pH (5.2) was recorded in full fat cheese while highest pH (5.39) was recorded in higher concentration of inulin. It is also confirmed by the findings of Katsiari and Voutsinas (1994) by their work on low fat feta cheese that the pH values of low-fat cheeses were slightly higher than that of the full-fat cheese throughout cheese ripening. It is depicted from the results that acidity of all cheese samples varied significantly due to various concentrations of dietary fibers used in preparation of low fat cheese. We can conclude that cheese samples having higher concentration of fiber had less acidity due to increased pH. pH and acidity are inversely related to each other. Romeih *et al*. (2002) also reported the same effect that due to increase in pH acidity of low fat white brined cheese decreased.

Texture analysis

Combination of sensory elements resulting from physical properties professed by sense of light and touch is defined as the texture (Fox *et al*., 2000). Texture is the most essential characteristic defining the product identity as well as attributes of food material resulting from combination of physical and chemical properties, especially for cheese (Buffa *et al*., 2001; Quigleya *et al*., 2011). Texture of cheese is greatly influenced by both compositional and processing parameters (Sattar *et al*., 2018; Wium *et al*., 2003). Hardness is the force necessary to bite entirely through sample when placed in the middle of molar teeth (Meullenet and Gross, 1999). The hardness of cheese reflects its chemical composition and the physicochemical state of constituents *i.e.* solid to fat ratio *etc*. Hardness also expresses the cheese macrostructure, indicating the existence of heterogeneities like granules connections in curd, fissures and cracks (Gunasekaran and Ak, 2003).

Figure 1 shows that by increasing the level of inulin and resistant starch cheese became harder as maximum hardness was found in cheese with more concentration. Maximum hardness was recoded in I_3 (2165.0 g) due to crystal formation, negative control (C -) have more hardness as compared to other treatments. All other factors depend upon hardness and force. Gumminess and chewiness dependent on hardness so they have the same trend as in hardness. Gumminess and chewiness increased by increasing the level of inulin and resistant starch.

The fat substituting property is based on its ability to stabilize the structure of aqueous phase which creates an improved creaminess. Creaminess is the interaction of inulin with caseinates. Long chain length reduces the solubility of inulin and results in the formation of microcrystals, so cheese become hard. Microcrystals interact with each other and forming small aggregates (Meyer *et al*., 2011).

The increase in hardness of cheese containing inulin was probably due to change in protein matrix compactness since addition of inulin increased water binding capacity of protein matrix. The results are supported by Sołowiej *et al*. (2015) who used inulin and whey protein polymers in low fat process cheese and found that by increasing level of inulin, hardness increases and melt-ability decreases.

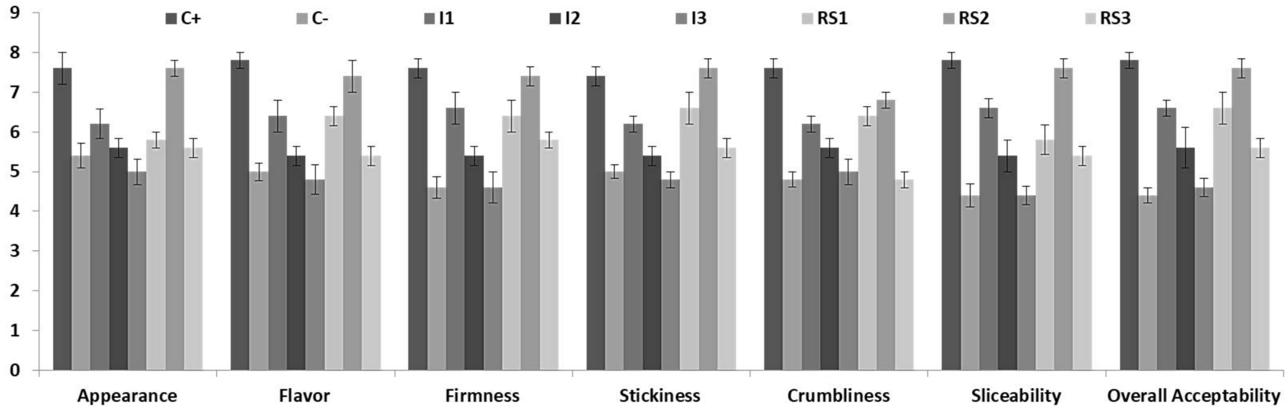

Fig. 2. Sensory evaluation of low fat cheddar cheese.

Decrease in fat with incorporated fat mimetic ingredients could increase protein water interaction enhancing adhesiveness in cheese. Gunasekaran and Ak (2003) conveyed that role of fat is as important that even if the moisture is higher in low fat Cheddar cheese, the texture will be hard due to more compact protein matrix with less open spaces (Murtaza *et al*., 2017). Oliveira *et al*. (2011) found that when level of starch increased in green Edam cheese, intermolecular interaction also increased, thereby creating a more compact three-dimensional matrix that effect the textural properties of low fat cheese. The fat substituting property is based on its ability to stabilize the structure of aqueous phase which creates an improved creaminess. Creaminess is the interaction of resistant starch with caseinates. Long chain length reduces the solubility of resistant starch and results in the formation of microcrystals, so cheese become hard. Microcrystals interact with each other and forming small aggregates (Chiavaro *et al*., 2007).

Functional analysis of low fat cheddar cheese

Quality of cheddar and processed cheese is determined for specific product claims and one of the determining factors for such claims is the melt-ability (Kuo *et al*., 2000). Melt-ability is term used to specify the range to which melted cheese flows and spreads on heating (Kuo *et al*., 2001; Sameen *et al*., 2008). It reflects the functional quality of cheese and is often defined by the consumers according to their requirement (Dorota and Pikul, 2009). The uniform melting quality of the cheese is a desirable property for consumer acceptance (Lefevere *et al*., 2000). Flow-ability is increase in diameter of the disc of cheese when heated at a specific temperature for a defined time or in other words it is the ability of melted cheese to flow. The factors responsible for flow-ability of cheese are the fat liquefaction, casein hydration and high degree of fat coalescence (Sattar *et al*., 2018; Fox *et al*., 2000).

Melt-ability and flow-ability also showed inverse relationship with levels of inulin and resistant starch. Melt-ability and flow-ability decreased by increasing their level and increasing hardness (Table III). Maximum melt-ability and flow-ability was noted in RS_1 as 68.00 mm and 24.70%, respectively. Yield calculation showed non-significant effect within 3 levels but significant effect as compared with control. Van Hekken *et al*. (2007) showed an increase in melt-ability in cheese by increasing fat content while Koca and Metin (2004) declared that decrease in fat causes decreased melt-ability.

Table III.- Functional analysis of low fat cheddar cheese.

Treatments	Melt-ability (mm)	Flow-ability (%)	Yield (%)
C+	61.00±1.67ab	22.90±0.31a	10.90±0.31a
C-	41.00±0.76c	12.88±0.35cd	8.76±0.16c
I_1	54.00±1.85b	18.70±0.42b	9.28±0.16b
I_2	44.00±0.98bc	15.30±0.51c	9.29±0.30b
I_3	39.00±1.10c	11.10±0.28d	8.52±0.29c
RS_1	68.00±0.94a	24.70±0.71a	10.15±0.12ab
RS_2	62.00±1.88ab	23.00±0.65a	10.47±0.22ab
RS_3	55.00±1.25b	19.40±0.40ab	10.12±0.17b

Values sharing same letters in one column are statistically non-significant from each other while values sharing different letters in one column are statistically significant from each other. For details of treatments, see Table I.

Sensory evaluation of low fat cheddar cheese

Mean scores for different sensory parameters are presented in Figure 2. Regarding the mean values the highest scores among the three levels of inulin for appearance (6.20±0.37), flavor (6.40±0.40), firmness (6.60±0.40), stickiness (6.20±0.20), crumbliness (6.20±0.20), sliceability (6.60±0.24) and overall acceptability (6.60±0.24) were awarded to I_1 having 0.5% inulin. All the three levels attained lesser score as compared with control (4% milk fat). In overall acceptability lowest score was given to I_3 (4.60±0.24). The addition of inulin as fat replacement improved the sensory characteristics of low fat cheese samples when added up to the level of 0.5% inulin. Increase in inulin levels in cheese samples decreases the scores awarded for various parameters. The lowest scores for all the parameters were awarded to the samples having 1.5% inulin. Regarding the mean values, the highest scores among the three levels of resistant starch for appearance (7.20±0.20), flavor (7.40±0.40), firmness (7.40±0.24), stickiness (7.60±0.24), crumbliness (6.80±0.20), sliceability (7.60±0.24) and overall acceptability (7.60±0.24) were awarded to RS_2 having 1.0% resistant starch. All the three levels attained less score as compared with control (4% milk fat). In overall acceptability least score was given to RS_3 (5.40±0.24).

When fat is removed, as in low fat cheese, casein plays greater role in texture development and texture become hard (Sattar *et al*., 2018; Mistry, 2001). The addition of inulin results in improved texture and appearance with score similar to the full fat product (Romeih *et al*., 2002). The addition of fat mimetics showed no substantial effect on flavor since they do not impart anything in flavor development. The results are supported by the study of Tiwari *et al*. (2014) that 4% inulin gave less acceptable sensory results than 2% inulin in low fat ice cream. Results are also in accordance with Kavas *et al*. (2004) who reported an improved cheese texture made with different fat mimetics than reduced fat control cheese. Increase in resistant starch levels in cheese samples decreased the scores awarded for various parameters as compared to the control sample. The lowest scores were awarded for all the parameters to the samples having 1.5% resistant starch. The addition of resistant starch results in higher appearance score similar to the full fat product (Murtaza *et al*., 2017; Romeih *et al*., 2002). The addition of fat mimetics showed no substantial effect on flavor since they do not impart anything in flavor development. Results are also in accordance with Kavas *et al*. (2004) who reported an improved cheese texture made with different fat mimetics than reduced fat control cheese.

CONCLUSIONS

It is concluded from the research that textural and functional characteristics with sensory attributes can be increased by addition of dietary fiber (inulin and resistant starch) as compared with negative control (low fat 2% fat level). Characteristics like full fat cheese can be attained by using inulin at 0.5% and resistant starch at 1% level without any adverse effects. In future combinations of inulin and resistant starch and combinations with other dietary fibers can be examined for more and more improvement in the product.

Statement of conflict of interest

The authors have declared no conflict of interests.

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