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Research Article

Effect of Inulin and Resistant Starch on Quality and Functionality of Low Fat Mozzarella Cheese Made from Buffalo Milk

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Keywords
Mozzarella cheese, Buffalo milk, Milk fat, Hydrocolloids, Texture, Meltability, Functional properties

Abstract | The demand of low fat cheese is increasing because of fat associated health risks. However, the low fat Mozzarella becomes hard and rubbery with reduced meltability and stretchability. The research was designed to explore the prospects of selected hydrocolloids to enhance the quality and functionality of low fat Mozzarella cheese from buffalo milk. Two hydrocolloids i.e. inulin (0.2, 0.4 and 0.6%) and resistant starch (0.5, 1.0 and 1.5) were added in Mozzarella cheese making from low fat milk (2% fat). The positive (4% milk fat) and negative (2% milk fat) control samples were also prepared for comparison. The Mozzarella cheese samples were analyzed for physico-chemical composition, functional properties, cheese yield and textural profile. The findings showed that there was a significant impact of added hydrocolloids (inulin and resistant starch) and their concentrations on the meltabilty, stretchability, hardness and yield of low fat mozzarella cheese. On the basis of functional parameters, cheese samples with inulin @ 0.4% and resistant starch @ 1.0% exhibited the best results. Hence, it was concluded that the addition of inulin @ 0.4% and/or resistant starch @ 1.0% as fat replacers revealed the exceptional prospects to produce low fat Mozzarella cheese without much compromising the quality and functionality.

Novelty Statement | In the current study, inulin and resistant starch were used in different proportions individually to assess their impact on the quality and functionality of low fat Mozzarella cheese from buffalo milk.

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Introduction

In Pakistan, buffalo population is the 2nd highest in the world and among milk production the buffalo milk is ranked second after cow milk (Huang *et al*., 2020). Pakistan has huge population of buffalo with 43.7 million heads and 39.50 million tons of milk (GOP, 2021-22). Buffalo milk is best suited for mozzarella around the world due to its composition and in Pakistan its availability in bulk makes it more convenient to use for mozzarella production (Murtaza *et al*., 2014; Qureshi *et al*., 2019).

In last couple of decades, the consumption of cheeses increased may folds. Among dairy products cheese is

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one of the most consumed items (Finnegan *et al.*, 2018). Popularity of cheese is increasing because of its nutritional, functional and organoleptic properties. There are hundreds of cheese varieties in the world which are classified on the basis of taste, flavor, method of preparation and nutritional profile (Walstra *et al.*, 2006). Milk preservation by transforming it to cheese started centuries ago. In cheese manufacturing process casein is coagulated and condensed into cheese with the help of coagulant (Murtaza *et al.*, 2014).

Mozzarella cheese one of the popular cheese with 32 percent of global cheese production. Due to unique functionality of mozzarella cheese it gained popularity around the world. It is widely used as a key ingredient on pizza toppings and sandwiches. Along with unique organoleptic and functional properties mozzarella is rich in minerals and vitamins. High intake of high fat dairy products may intensify the danger of cardiovascular maladies (Nagai *et al.*, 2013; Raza *et al.*, 2017). With changing eating habits of the masses the demand of pizza and the mozzarella is increasing. Escalation in pizza consumption is linked with the increase cardiovascular maladies (Kindstedt, 2004). Decreasing fat in mozzarella is of without influencing the characteristics of the cheese is of great importance. Fat play a key role in the cheese matrix (McClements and Demetriades, 1998) and minimizing the fat of mozzarella while having minimalistic impact on the functional characteristics is of high importance for the industry and the consumer (Palatnik *et al.*, 2017).

The functional and organoleptic traits of cheese are dependent on the composition of the cheese. Minimizing fat in the cheese affects its composition and with ultimate impact on the functional characteristics. The reduction in fat contents of the cheese make it hard and rubbery with objectionable texture and aroma (Romeih *et al.*, 2002; Roger *et al.*, 2010). While plummeting the fat in cheese certain gums and carbohydrates based fat replacers are used to minimize the drastic effect fat reduction on cheese. These fat replacers interact with water to form gel to improve the functional parameters of the cheese (Koca and Metic, 2004; Madadlou *et al.*, 2007). Starches, inulin, gums and other polysaccharides are being employed to enhance the textural profile and functional parameters of the food products (Simeone *et al.*, 2004).

Certain ingredients like fat replacers and fat mimetics are being used to alleviate the impact of fat reduction. These ingredients can trap water and form a gel to enhance the functionality of the Mozzarella cheese (Koca and Metic, 2004; Madadlou *et al.*, 2007). Various types of polysaccharides like guar gum, starch, inulin, and exopolysaccharides producing starter cultures are being used to improve the texture and functionality of food products (Simeone *et al.*, 2004; Perry *et al.*, 1997).

Inulin is a carbohydrates based polymeric compound having fructan as basic unit. It is widely used in various food products and is safe to use in dairy products as food ingredient in varying amounts as stabilizers and fat replacers (Chaito *et al.*, 2016). In food products inulin is used in food products to achieve numerous desirable results as enhancement of organoleptic properties and improving the texture profile of food products (Karimi *et al.*, 2015). It acts as prebiotics in foods and enhance the proliferation of intestinal micro flora. The acceptable results regarding textural improvement and organoleptic improvements can be achieved when inulin is use in dairy product in the range from 2-10 percent (Karimi *et al.*, 2015). Inulin like other starches possesses outstanding water binding capability that inhibit syneresis in cheese and enhance the functional properties (Fadaei *et al.*, 2012).

In food products starches play multiple roles like acid hydrolysis and cross linkages in protein matrix which make then best suited to be used as substitutes (BeMiller and Whistler, 1996). In the production of low fat cheese starches can be used as fat replacers as cross linking of starches and protein molecules helps to mitigate the drastic impact of decreasing fat in cheese and improve the functionality of the cheese (Noronha *et al.*, 2007). Resistant and natural starches can be employed in cheese with low fat to improve the textural behavior and functionalities (Mounsey and Riordan, 2008). Keeping in view the properties of hydrocolloids, the present work was intended to evaluate the impact of selected hydrocolloids (resistant starch and inulin) in different proportions on quality and functional properties of low fat buffalo milk Mozzarella cheese.

Materials and Methods

Raw materials

Buffalo milk was obtained from a local dairy farm near in Sargodha. Thermophilic bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* sp. *bulgaricus*) and milk coagulant enzyme chymosin along with fat replacer i.e., inulin and resistant starch were used for the preparation of Mozzarella cheese

Cheese manufacturing and analysis

Buffalo milk was first standardized at 4% and 2% milk fat. Mozzarella was manufactured as per the procedure described by Zisu and Shah (2007) with little modification for the addition of inulin (0.2 %, 0.4% and 0.6%) and resistant starch (0.5%, 1.0% and 1.5%) from low fat milk (2% fat). The positive (4% milk fat) and negative (2% milk fat) control (cheese samples) were also manufactured as per treatment plan (Table 1). The mozzarella samples were stored at 6-8°C and analyzed for physico-chemical, functional and sensory parameters.

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Table 1: The detail of cheese samples (treatments) prepared.

Treatments	Fat level (%)	Hydrocolloids level (%)
Positive Control (C)	4	-
Negative Control (C')	2	-
Inulin (I)	2	0.20, 0.40, 0.60
Resistant Starch (RS)	2	0.5, 1.0, 1.5

Physico-chemical composition

The physico-chemicals analysis of mozzarella cheese was carried out for all the selected samples. The fat percentage of the mozzarella was carried out as per the method elaborated by Marshall (1992). The moisture percentage of the cheese samples was done with the help of oven drying technique at a temperature of 105±5°C (AOAC, 2000; Method No. 926.08). The Kjeldahl apparatus was used to estimate the crude protein contents of the cheese samples (Lynch and Barbano, 1999). The ignition method using the muffle furnace was used to find out the ash contents in mozzarella (AOAC, 2000; Method No. 935.42). Metzger *et al.* (2000) method of determination of calcium contents in the cheese was employed on the cheese samples.

The electrode pH meter (Hanna, HI-99161) was used as per method of Ong *et al.* (2007) to determine the pH of the cheese which an important parameter in cheese. At ambient temperature of 25°C, 20g of cheese sample was incorporated in 12ml of distilled water then the pH was determined using pH meter. The acidity percentage of cheese was determined by titration of the filtrate of cheese sample using the procedure described in AOAC (2000) method No. 920.124. In this method 10 ml little warm water was taken in beaker and 1g cheese was mixed in it thoroughly then the solution was filtered. For titration 0.1 N NaOH solution was used with phenolphthalein indicator with light pink color as end point of the titration.

Functional analysis of mozzarella cheese

The mozzarella samples were subject to analysis in for melt-ability, stretch-ability, cheese yield in triplicate to check the impact of inulin and resistant starch on the functionality of cheese.

Meltability

Cheese samples were inserted in glass tube of 24mm with known length as per method described by Zisu and Shah (2007). With the help of a plunger the cheese samples were inserted in open end of the tube and the length of cheese in tube was measured and then the samples were kept for four hours at a temperature of 4°C. The samples were then given heat treatment at 110 °C for a period of 100 minutes. The melted length of cheese in the tube was recorded using vernier caliper.

Stretchability

The stretch-ability of mozzarella samples were recorded following the procedure described by McMahon *et al.* (2009). The fork was inserted in the method mozzarella samples and then mozzarella samples were stretched by stretching the fork until the breakage of the cheese strands. The stretch-ability was measure in centimeters.

Cheese yield

The percentage yield of mozzarella cheese was calculated as per standard method described by Sipahioglu *et al.* (1999).

Texture analysis

The samples of the mozzarella were subjected to textural profile analysis to determine the textural variations in the cheese. TA-XT2i Plus Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK) was used for texture profile analysis (Zisu and Shah, 2007).

Statistical analysis

The study results were illustrated in triplicate and Minitab 19.1 software was employed to statistically analyze the study results using completely randomized design. The variance and level of significant were estimated using one-way ANOVA (Montgomery, 2013).

Results and Discussion

Reducing fat in mozzarella affect its sensory and functional attributes along with rheological properties. The core objective of this study was to highlight the best dose of inulin and resistant starch that can alleviate the drastic effects of fat reduction on mozzarella. The low fat mozzarella with added inulin and resistant starch was compared with the full fat mozzarella at 4% milk fat and 2% mozzarella without any addition of fat replacer.

Physico-chemical analysis

The addition of inulin and resistant start in the samples of low fat mozzarella has significant ($P<0.05$) impact on the physico-chemical properties of the cheese. The highest moisture was determined in the low fat mozzarella cheese with 0.4% inulin as fat replacer. Moisture is one of the main contributor of the functionality of mozzarella because of its role as plasticizer in protein matrix (Mcsweeney and Souse, 2000). The positive control sample retained lesser moisture than the negative control and the low fat mozzarella samples with added fat replacers. For the protein contents lowest protein was present in positive control sample and highest protein was determined in sample with 0.4 percent inulin and 1.0 percent resistant starch, respectively. Highest fat was determined in positive control sample while lowest was present in negative control sample. In treatment samples among inulin I2 sample showed higher fat while among resistant starch RS2 sample revealed higher fat percentage.

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Non-significant impact treatments on ash percentage was noted among all treatment samples. Ash percentage denotes the inorganic material present in the cheese that can be estimated through muffle furnace at elevated temperature (Kirk and Sawyer, 1991). It was evident from study results that inclusion of fat replacers exhibited non-significant results on cheese mineral contents which are in agreement with the findings reported by Sattar *et al.* (2018). pH has a definite effect on the cheese during manufacturing. The values of pH showed non-significant impact of inulin and resistant starch dosage on the pH values of the cheese samples. These results are in line with the findings of Murtaza *et al.* (2022). The researchers Shendi *et al.* (2010) publicized no alteration of pH values of cheese with the use of arabic gum as fat replacers. In cheese fermentation of milk sugar resulted in formation of acids that has profound impact on the shelf life and structure of the cheese (Widyastuti *et al.*, 2014; Ceylan *et al.*, 2003). The fat reduction and addition of inulin and resistant starch did not affect the acidity of the cheese.

Functional parameters of low fat mozzarella cheese

Meltability and stretchability properties of the cheese represents the functional properties of mozzarella. These properties are the ones which are highly affected by reduction of fat in cheese. The meltability has been reported in published literature in terms of cheese particles presence in the form of stream embedded in liquefied mass in uniform manner (Kindsted and Fox, 1993). Fat substance in cheese involved the linkages of protein-protein as well as protein-water interaction which serves as the principal factor to govern meltability of Mozzarella cheese (McMahon *et al.*, 1999).

It has been shown by the results that hydrocolloids in terms of different levels significantly ($p<0.01$) influenced the low fat mozzarella meltability. The mean values of meltability in various low fat mozzarella differed between 45.10 to 49.1 mm for inulin and 43.20 to 51.10 mm for different level of resistant starch Tables 2 and 3. The results showed that the peak level of metlability was found from the cheese manufactured with 4% milk fat and lowest level of meltability was noticed with the cheese having 2 % milk fat. Consequences of the current investigation in regards to cause decreases in the Mozzarella cheese meltability with corresponding decrease of fat levels as reported by Rudan *et al.* (1999) who described that Mozzarella cheese exhibited decreases in case of meltability as the fat substance of the cheese diminished. Lu *et al.* (2017) additionally revealed converse connection between fat decrease and cheese meltability. Variety in cheese meltability regarding various kinds of hydrocolloids may be because of their diverse synthetic piece and conduct during cheese liquefying. It was also reported by Oliveira *et al.* (2011) that increased levels of guar gum in green Edam cheese caused strengthening of intermolecular connection, accordingly making a compact and conservative 3-D network that caused decreases in water retention in case of cheese curd. In order to make the Mozzarella cheese soften, fat globules are required in terms of continuous fat globules combination. However, when the hydrocolloids level surpassed after certain levels, and these also may lead to interference with those of fat globules during dissolving. Besides, it was also reported that hydrocolloids exhibit profound water network; subsequently the level showed diminishing tendency and to improve water mass transfer within the cheese framework to interact with the fat molecules stream during liquefying of cheese.

Stretchability is defined as the casein inclination to support it after application of consistent pressure to the cheese. After the cheese extension, the casein atoms interface and increased the malleability (Lucey *et al.*, 2003). Low fat mozzarella cheeses exhibited significant stretchability ($p<0.01$) which was influenced by various levels and kinds of fat replacers used. Results showed that mean stretchability determined in various mozzarella cheeses varied from 38.4 to 49.78 cm.

Table 2: Physico-chemical analysis of low fat Mozzarella cheese.

Treatments	Moisture %	Protein %	Fat %	Ash %	pH	Acidity %
C	46.34±1.06e	24.17±0.24d	24.60±0.29a	3.31±0.01a	5.20±0.02a	0.930±0.025a
C’	49.94±0.85c	28.04±0.30c	10.90±0.20d	3.32±0.08a	5.21±0.04a	0.924±0.017a
I1	51.10±0.80a	29.82±0.86ab	12.70±0.18c	3.35±0.07a	5.22±0.02a	0.921±0.016a
I2	51.60±0.94a	30.08±0.84a	13.40±0.11b	3.38±0.10a	5.26±0.02a	0.925±0.012a
I3	50.61±0.14b	29.96±1.05ab	13.10±0.21b	3.29±0.04a	5.23±0.03a	0.924±0.017a
RS1	49.04±0.89cd	29.65±0.45b	12.06±0.20cd	3.31±0.06a	5.25±0.02a	0.917±0.009a
RS2	49.77±0.94c	30.07±0.21a	12.66±0.30c	3.35±0.02a	5.28±0.01a	0.929±0.012a
RS3	48.07±0.94d	29.08±0.04bc	12.00±0.30cd	3.30±0.02a	5.21±0.01a	0.925±0.012a

The values shown in column with similar statistical lettering exhibited non-significance at P>0.05. C = control with 4% fat in milk; C’ = negative control with 2% fat in milk; I1 = mozzarella cheese low fat (MCLF) with 2% milk fat and 0.2 % inulin; I2= mozzarella cheese low fat (MCLF) with 2% milk fat and 0.4 % inulin; I3= mozzarella cheese low fat (MCLF) with 2% milk fat and 0.6 % inulin; RS1 = mozzarella cheese low fat (MCLF) with 2% milk fat and 0.5 % resistant starch; RS2= mozzarella cheese low fat (MCLF) with 2% milk fat and 1.0 % resistant starch; RS3= mozzarella cheese low fat (MCLF) with 2% milk fat and 1.5 % resistant starch.

Table 3: Effect of Inulin and Resistant starch on stretch-ability, melt-ability and yield of Mozzarella cheese.

Treat-ment	Stretchability	Meltability	Yield
C	49.78±1.097a	59.70±1.270a	14.58±0.266a
C'	38.40±0.473c	47.20±1.403bc	10.90±0.202b
I1	42.20±1.299bc	49.10±1.386bc	11.61±0.388b
I2	44.71±1.686ab	51.20±1.050b	13.30±0.231a
I3	41.10±1.495bc	45.10±0.693c	11.91±0.352b
RS1	40.10±1.195bc	43.20±1.905b	11.72±0.092c
RS2	44.70±1.397ab	51.10±1.039ab	12.91±0.115b
RS3	39.50±1.143bc	47.10±2.252b	11.23±0.150cd

The values shown in column with similar statistical lettering exhibited non-significance at P>0.05. C= Control with 4% fat in milk; C'= negative control with 2% fat in milk; I1 = mozzarella cheese low fat (MCLF) with 2% milk fat and 0.2 % Inulin; I2=mozzarella cheese low fat (MCLF) with 2% milk fat and 0.4 % inulin; I3= mozzarella cheese low fat (MCLF) with 2% milk fat and 0.6 % inulin; RS1= mozzarella cheese low fat (MCLF) with 2% milk fat and 0.5 % resistant starch; RS2= mozzarella cheese low fat (MCLF) with 2% milk fat and 1.0 % resistant starch; RS3= mozzarella cheese low fat (MCLF) with 2% milk fat and 1.5 % resistant starch.

In the current examination, decrease in stretchability with decline in fat level are very much upheld by the discoveries of Sattar *et al*. (2018) who announced that fat decrease in cheese brought about helpless stretch trademark. Anyway, these discoveries are not in accordance with Kindsted and Fox (1993) who revealed that stretchability is contrarily connected with the degrees of fat. Ahmad *et al*. (2020) tracked down that low fat cheese extended indeed however the stretch quality in low fat cheese was low (more sinewy, slim strands, not so much flexible but rather more fragile) than those shaped by the high fat cheese. Distinctive extending conduct in LFMCs fabricated by utilizing various sorts and level of hydrocolloids is because of the explanation that cheese extending includes protein combination to form framework. Any material having the incongruency with protein, for example, the presence of hydrocolloids in an adequate amount; these resulted in meddling of the protein combination measure during the extension of cheese network and eventually diminished its qualities. It was evident from the results that influence of hydrocolloids levels exhibited significant level ($p < 0.01$) on yield of low fat cheese. Yield of cheese values was found in range of 10.4-14.01% as presented in Figure 4.1.3. It was obvious from results that various hydrocolloids utilization in assembling of LFMCs which led to cheese yield expansion as all cheese hydrocolloids contained low fat mozzarellas display better return (11.61 to 14.58%) when contrasted with C/(10.40 %) for example low fat mozzarella made without expansion of hydrocolloids. It was evident from the results that fat content decreased cheese milk.

Consequences of the current investigation in regards to diminish in cheese yield with decline in fat degree of cheese milk as reported by Rudan *et al*. (1999). Likewise, fat substance and milk casein are subjected to cheese curd maintenance which was consistent with the cheese yield (Sahan *et al*., 2008). The rise in low fat yield cheese was attributed to the fat mimetics owing to water restriction (Mistry, 2001).

Textural parameters of low fat mozzarella cheeses

The term texture of food products can be defined as is combination of sensory features which are the result of physical properties of the product avowed by the senses of light and touch (Fox *et al*., 2000). The texture is one of the most crucial properties of the cheese which is the result of both physical and chemical parameters of the cheese (Quigleya *et al*., 2011). In textural analysis parameter like hardness and chewiness were analysis and described in the section below.

Hardness

The term hardness is defined as the force required to get a deformation in a given sample when it is place between two molars is called hardness (Nateghi *et al*., 2012). In mozzarella cheese the hardness of cheese is dependent on the distribution of fat and water molecules in the protein matrix of the cheese.

The values of the Table 3 showed that the harness varies from 1156 to 2876. It is clear from the results that highest hardness was observed in sample of negative control (C') with reduced fat and no addition of any hydrocolloids and the lowest hardness was observed in the sample having full fat i.e. C. It is also evident from the results that hardness of low fat mozzarella cheese decreases with the addition of inulin and resistant starch. The presence of high fat contents in cheese proved to be the weaker points in the protein matrix which make is less hard than the cheese matrix without fat. Awad *et al*. (2005) analyzed that fat droplets in protein matrix allow slippage of protein matrix which allow deformation of structure without breakage. In low fat cheese the slippage is less and cheese having high hardness. When fat is removed the protein matrix of the cheese become compact and cheese become harder (Zisu and Shah, 2007). High fat in cheese play the role of lubricant and cheese behave softer (Koca and Metin, 2004).

As fat replacer, addition in cheese act as filler as replacement of fat and increase water holding capacity of the cheese which decrease hardness of the cheese. The volume of cheese with added hydrocolloids becomes higher than the low fat mozzarella cheese without any addition of hydrocolloids. With the increase of the volume the moisture to protein ratio of the cheese is also increased and less force is required for deformation of cheese (Sattar *et al*., 2018). In previous studies it was found out the addition of hydrocolloids reduced the hardness of the cheese (Zisu and Shah, 2005; Koca and Metin, 2004).

Table 4: Effect of inulin and resistant starch on hardness and chewiness of Mozzarella cheese

Treatments	Hardness	Chewiness
C	1156.0±9.81m	683.0±9.66k
C'	2876.0±64.66a	2296.8±44.82a
I1	1865.0±15.01d	1221.2±36.17de
I2	1851.0±21.94de	1180.2±26.10def
I3	1875.0±5.20d	1302.2±19.77d
RS1	1821.0±40.99def	1238.1±29.59de
RS2	1812.0±27.71def	1139.8±18.49gh
RS3	1841.0±30.98def	1278.1±29.59de
Means	1887.1±104.85A	1292.4±102.64A

The values shown in column with similar statistical lettering exhibited non-significance at P>0.05. Interaction means were shown by small lettering while overall mean was shown by the capital lettering.

The results of current study are in accordance with the finding of the Sipahioglu *et al.* (1999) who found that low fat cheese made with addition of tapioca starch exhibit less harness that the control sample with reduced fat. Ahmad *et al.* (2020) and Sattar *et al.* (2018) found that low fat mozzarella cheese with added hydrocolloids showed less hardness that the low fat cheese control sample.

Chewiness

Chewiness can be defined as the time lapse required to grind the sample at a level that it can easily be swallowed (Zoon, 1992). The mean values of the chewiness of the low mozzarella cheese are displayed in Table 3. The value of the chewiness shows that the chewiness varied from 683 to 2296. The full fat mozzarella cheese showed lowest value of chewiness among samples while reduced fat cheese without any treatment showed significantly higher value of chewiness (2296). In mozzarella cheese when fat is reduced the in response to reduced fat the mozzarella cheese exhibit rubbery and tough texture which is harder to chew. While using inulin treatment I2 having 0.4 percent inulin showed better results with 1180.2 chewiness and using different levels of resistant starch RS2 in which 1.0 percent resistant starch performed better than other treatments having different levels of resistant starch. Sattar *et al.* (2018) found that when fat is removed the protein matrix became compact and hard to chew. The treatments showed chewiness in the range 1039 to 1302. The findings of the current study are in line with the findings of Murtaza *et al.* (2017) who also reported that addition of hydrocolloids decreased the chewiness of the reduced fat cheese samples. Hardness of cheese is also linked with the chewiness as harder cheese is difficult to chew than the softer cheese (Beal and Mittal, 2000). Nateghi *et al.* (2012) defined chewiness as the no of chomp needed for a certain quantity of sample to decrease its consistency suitable for swallowing

Conclusions and Recommendations

It was concluded that low fat Mozzarella can be manufactured successfully using different levels of inulin and resistant starch having significant impact on the functional and textural properties of mozzarella. The use of inulin @ 0.4% and resistant starch @ 1% in mozzarella cheese with 2% milk fat showed the results closer to the full fat cheese. It is recommended that some other hydrocolloids may also be tested for low fat cheese and other dairy products in future studies on similar lines.

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Ethics approval consent to participate

Not applicable.

Conflict of interest

The authors affirmed no conflict of interest.

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