SHORT COMMUNICATION



Process standardization for rennet casein based Mozzarella cheese analogue

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Abstract A process for manufacture of Mozzarella cheese analogue (MCA) using rennet casein and plastic cream as protein and fat sources respectively was standardized. The formulation comprised of 25% plastic cream (72% fat), 27% rennet casein along with 3% trisodium citrate as emulsifying salt, 2% maltodextrin as binder, 0.55% lactic acid as pH regulator, 1% common salt for seasoning, 1% Mozzarella cheese bud as flavouring and 40.4% water. The process involved (a) dissolving the dry mixture of casein, maltodextrin, flavouring and common salt in hot emulsifying salt solution, (b) incorporation of half the quantity of acid solution in casein-maltodextrin dough, followed by addition and emulsification of plastic cream, and (c) addition of remaining half of the acid solution and heating the mass to 80 °C until a plastic cheese mass was obtained. The analogue was shaped in ball form, cooled and packaged in polyethylene bag. The MCA conformed to the PFA requirements for pizza cheese and had all the requisite baking characteristics expected of pizza cheese topping.

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K. D. Aparnathi Department of Dairy Chemistry, S.M.C. College of Dairy Science, Anand Agricultural University, Anand 388 110 Gujarat, India **Keywords** Mozzarella cheese · Analogue · Rennet casein · Plastic cream · Process standardization · Composition

Cheese analogues also referred to as cheese substitutes or imitations, are the products that partly or wholly substitute or imitate cheese and in which milk fat, milk protein or both may be partially or wholly replaced by non-milk based components, principally of vegetable origin (Fox et al. 2000). Analogue cheese products are made using techniques similar to those used for processed cheese manufacture with the aid of heat, mechanical shear and emulsifying salts. Analogue cheese products are categorized as: (a) dairy, (b) partial dairy, or (c) non-dairy analogues (Shaw 1984). Though cheese analogues are produced commercially in advanced countries, the information pertaining to their formulation and method of manufacture are invariably patented (Rule and Werstak 1978; Bell et al. 1980; Finnie and Olsen 1998) making such information inaccessible to the scientific community. The cheese variety which has been imitated the most is Mozzarella cheese due to the popularity of pizza world over, in which cheese is the key ingredient (Shaw 1984). The advantages in making cheese analogue include use of cheaper raw materials (casein and maltodextrin instead of normal milk solids), ease of manufacture, fewer equipment (blender, processing kettle) and manpower requirements.

Mozzarella cheese analogue (MCA) having satisfactory application on pizza pie has been made using acid casein, vegetable oil blend and other functional ingredients. The MCA had 15.2% fat, 20.1% protein and 0.9% salt (Jana and Upadhyay 2001). MCA has been made employing rennet casein and vegetable oil/fat using disodium phosphate (DSP) (O'Malley et al. 2000) and mixture of DSP and tri-sodium citrate (Mounsey and O'Riordan 2008a) as emulsifying salts. Thus, keeping in view the potential of MCA, a study was planned to standardize a functionally effective formulation and evolve a suitable technique for its manufacture using rennet casein powder as the protein source and plastic cream as the fat source, along with other requisite additives.

Materials and methods

Fresh plastic cream (\geq 72% fat) was obtained by separating whole milk, while powdered rennet casein was prepared from fresh skim milk at 'Anubhav Dairy'—an experimental dairy plant of the university. Tata brand common salt was obtained from local market. Maltodextrin (Indian Maize and Chemicals Ltd, New Delhi), tri-sodium citrate (TSC), dihydrate (LR) (Qualigens Fine Chemicals, Mumbai), di-sodium hydrogen orthophosphate, dihydrate (AR) (Samir Tech Chem Pvt Ltd, Vadodara), lactic acid (S D Fine Chemicals Ltd, Boisar) and Mozzarella cheese bud flavouring (Duke Thompson's India Ltd, Indore) were obtained.

Edible grade rennet casein from fresh mixed (cow and buffalo) skim milk was prepared as per the procedure of Webb and Whittier (1970) using Maxiren (Fungal rennet, 1,800 IMCU/g strength, Duke Thompson's India Ltd, Indore) as coagulant. The casein was dried to about 9.5% moisture in a forced-draft tray drier (Yorco Sales Pvt Ltd, New Delhi), using hot (58 ± 2 °C) air. Hammer mill (Magnum Gold F-826; Swastik Sales Corp, Vithal Udyognagar) was used to mill the dried casein. The ground casein was sieved through IS sieve of 355 µm to obtain desired sized particles.

On the basis of preliminary trials, the method of preparation of MCA from rennet casein and plastic cream was standardized. Hobart food processor (Model N 50, Hobart Corp, Canada) operating at 3 speeds was used to blend the ingredients. During the experimentation, 500 g of MCA was prepared for each treatment. The experiment was replicated 6 times.

The cheese analogues and rennet casein were analyzed for total solids using Mojonnier milk tester (MIF 1959), fat by Van Gaulik method (BIS 1979), protein by micro-Kjeldahl method (Jana and Upadhyay 2001), ash (BIS 1961), pH and salt (Patel et al. 1986).

The cheese analogue, as pizza topping, was sensorily assessed by a panel of 6 judges. Seventy grams of shredded cheese was topped on pizza base (~15 cm diameter, 1.5 cm thick) and baked in a forced-draft oven at 230 °C, until the cheese melted completely (6–8 min). Maximum score of 10 was used for sensory attributes like appearance (including fat leakage, browning), flavour, melting, stringiness and chewiness in a manner similar to the liking for such attributes in 9-point Hedonic scoring. The final sensory score for each cheese was given out of 50. Melting was sensed by visual appearance of baked pizza with cheese

topping. While taking a bite of pizza with cheese topping, the uniform thickness of melted cheese pulled by the teeth was noted as melting criteria. Stringiness was apparent while putting a bite on cheese topped on pizza pie and pulling back the pizza pie which resulted in cheese being stretched. Chewiness was felt during mastication of cheese when chewed by molar teeth.

MCA was analyzed subjectively for shredding ability using a manual shredder. Meltability was tested by Schrieber melt test (Park et al. 1984), fat leakage by the method of Breene et al. (1964) and stretchability using stretch tester (Hi-TST-E-Cheese Machine, Hiranya Instruments, Vadodara). Meltability of cheese was expressed as arbitrary value based on the numbers assigned to the concentric circles on the cheese disc. Higher number indicates greater melt diameter and hence better meltability of cheese.

Results and discussion

To obtain desired fat content in cheese analogue, it was thought appropriate to use dairy cream (instead of vegetable oil) since it gives superior flavour and contains fat in emulsified form. High fat ($\sim 72\%$) cream (having lower water than reduced fat creams) was chosen so that the quantity of water that can be used in the formulation is sufficient to dissolve the rennet casein. The quantity of plastic cream that is to be added was based on obtaining fat content in MCA which could satisfy the fat on dry matter (FDM) content (minimum 35%) as prescribed by PFA (2006) for pizza cheese. This necessitated using 25% plastic cream to obtain a cheese with 21.8% fat and 39.1% FDM (Table 1).

Natural Mozzarella cheese contains predominantly casein protein. Since natural Mozzarella cheese employs rennet for curd formation, it was presumed that use of rennet casein in cheese analogue might contribute to the functional properties expected from rennet based cheese. In the present study, dried rennet casein having 9.6% moisture, 1.3% FDM, 89.3% protein (on dry matter), 8.8% ash (on dry basis) and pH of 6.7 was used. Rennet casein was used in the formulation at 27% level, which

Table 1 Proximate composition (% w/w) of Mozzarella cheese analogue	Moisture	44.3
	MFFS ^a	56.6
	Fat	21.8
	FDM ^b	39.1
	Protein	26.2
	Salt	1.2
	Ash	5.5
^a Moisture-in-fat-free substances,	pН	6.6

^D Fat-on-dry matter, n=6

resulted in MCA possessing 26.2% protein (Table 1). Natural milk based Mozzarella cheese is reported to contain 50.6% moisture, 18.4% fat, 28% protein, 1.2% salt and 2.5% ash (Jana and Upadhyay 2003).

It was presumed that use of maltodextrin in the formulation might help in reducing the firmness of caseinbased cheese analogue, rendering the cheese moderately chewy and probably improve meltability. Cheese analogues made using casein alone are reported to give unacceptably strong and rubbery product (Lee and Son 1985). Moreover, incorporation of maltodextrin will improve the solids content thereby decreasing the moisture content of cheese, which probably improves shredding ability. Starch derivative is also cheaper than milk protein. Some of the starch derivatives used in analogue cheeses include native starch, boiled starch, pre-gelatinized starch, and maltodextrin (Finnie and Olsen 1998; Mounsey and O'Riordan 2008a, b; Montesinos-Herrero et al. 2006). Preliminary trials were conducted to find out the influence of using 2, 3 and 4% maltodextrin in the formulation. It was found that 2% maltodextrin gave sensorily best MCA with desired stretch quality; higher levels (3 and 4%) led to impairment in the stretch character.

Acidulants have a wide variety of roles in cheese such as preservation, texture development, pH adjustment and flavour contribution. Use of citric, lactic, adipic or malic acids have been reported for adjusting the pH of imitation cheeses (Fox 1978; Bell et al. 1980). Desired pH is critical in deciding the functionality of cheese analogue. Addition of lactic acid gave good result in the manufacture of directly acidified Mozzarella cheese (Jana and Upadhyay 2003). Hence, lactic acid was selected for adjusting the pH of cheese curd, at which the cheese analogue had desirable baking (stretch, melt) characteristics (Table 2). Lactic acid

 Table 2
 Baking characteristics of Mozzarella cheese analogue when used as pizza topping

Baking properties $(n=6)$	
Shredability (subjective)	Very good
Melting time in oven at 230 °C, min	6.2
Schreiber meltability, Arbitrary value	4.3
Fat leakage, cm ²	7.1
Stretch test, cm	34.0
Sensory rating as pizza topping	
Appearance ^a	7.7
Flavour ^a	8.0
Melting ^a	8.2
Stringiness ^a	8.2
Chewiness ^a	8.1
Total score ^b	40.2

Out of ^a 10 and ^b 50, n=6 panelists

was used at a level of 0.55% in the MCA formulation, and it yielded a product having pH of 6.6 (Table 1).

Cheese analogues are produced by techniques used for the production of processed cheese. Emulsifying salts help in solubilizing the rennet casein. Emulsifying salts acts on protein and influence emulsification of fat (Cavalier-Salau and Cheftel 1991) and meltability of cheese (Shimp 1985). In processed cheese, a maximum of 4% of emulsifying salts is permitted by PFA (2006). Hence, TSC alone and in combination with DSP in 1:1, 1:2 and 2:1 proportions was tried out at 3% by weight in the formulation. This was attempted since a blend of TSC and DSP (1:1.7) was useful in obtaining MCA based on acid casein (Jana and Upadhyay 2001). Neither of the combinations of TSC and DSP gave satisfactory quality cheese analogue; the problem faced was 'surface crust' formation in baked cheese topped on pizza. However, TSC when used alone at 3% level in the



Fig. 1 Standardized process for manufacture of Mozzarella cheese analogue (1 kg)

formulation vielded MCA with desired melt and stretch properties (Table 2).

Natural Mozzarella cheese is reported to have a salt content of 0.8-1.2% (Jana and Upadhyay 2001). To obtain low salted MCA, common salt was added at 0.9, 1.0 and 1.1% (w/w) levels in the formulation. Of these, 1% salt gave the desired saltiness in the cheese.

Cheese analogue requires cheese flavourings (starter distillates, enzyme modified cheese) to attain the desirable flavour of cheese. In the present study, 'Mozzarella cheese bud flavouring' was employed at the rate of 0.5, 1 and 1.5% (w/w) in the formulation. Adequate cheese flavour was perceived in MCA when cheese bud flavouring was added at 1% level.

Water is not only necessary for dissolving dry ingredients (casein, maltodextrin, salt, flavour, emulsifying salt) in the manufacture of cheese analogue, but it determines the moisture content of product. Low-moisture part skimmed Mozzarella cheese contains 45-50% moisture (Jana and Upadhyay 2003). Once the quantum of other ingredients as discussed above was fixed, the quantity of water required in the cheese formulation was obtained by difference. The water used in the MCA formulation (40.4%; Fig. 1) led to MCA having average moisture content of 44.3%. PFA (2006) permits a maximum of 54% moisture in 'Pizza cheese'. Based on the above findings, the standardized formulation for MCA based on rennet casein and plastic cream arrived at in the experimentation is shown in Fig 1.

The process of manufacturing MCA was first standardized based on preliminary trials and then the proportions of ingredients used in the formulation were selected. Two

aspects were standardized (i) mode of incorporation of acid. and (ii) the processing temperature to be used for obtaining MCA with desired baking properties.

Table 3 shows the influence of acid incorporation in two installments viz., half of the acid solution (lactic acid: water 1:10 w/v) incorporated in slurry comprising of rennet casein, maltodextrin, salt and emulsifying salt, followed by incorporating the remaining half acid solution to the fatincorporated dough. This mode of acid incorporation gave superior quality (especially baking properties) MCA compared to other method of acid incorporation wherein half of the acid solution was added to the water containing emulsifying salt, followed by addition of the remaining acid to the dough comprising of rennet casein, maltodextrin, salt and flavour (Table 3). The melting as well as stretch character of the MCA was superior when acid was added in two installments, first to casein slurry devoid of fat and secondly following fat incorporation. Hence, this mode of pH adjustment was chosen (Fig 1).

During final heating of the casein dough after fat (cream) incorporation, it was necessary to process the dough to pasteurize the cheese and to develop the stretch character. It was decided to raise the temperature of dough to 78, 80 and 82 °C. It was found that the yield of cheese mass decreased with increase in the temperature of processing; the yield was 87.5, 86.5 and 85% respectively when cheese mass was heated to 78, 80 and 82 °C. The meltability of cheese analogue was best (Schreiber melt 4.8) when cheese mass was heated to 80 °C; the meltability values were lower when either 78 °C (4.5) or 82 °C (4.6) was adopted (Table 3). The somewhat over-desiccation in cheese mass heated to 82 °C might have

Table 3 Process standardization for Mozzarella cheese analogue	Process parameters		
	Acid incorporation in 2 stages	Effects	
	(a) First half of acid addition to emulsifying salt solution and second half to dough comprising of rennet casein, maltodextrin, salt and cheese flavour.	pH of MCA=6.6 Schreiber melt ^b =4.1 Stretch test=37 cm	
	(b) First half of acid addition to rennet casein slurry containing maltodextrin, salt and cheese flavour, while second half to fat (cream)-incorporated dough.	pH of MCA=6.6 Schreiber melt ^b =4.8 Stretch test=45 cm	
	Final cheese processing temperature		
	(a) 78 °C	% Yield of cheese ^a =87.5 Schreiber melt ^b =4.5	
	(b) 80 °C	No browning on pizza. % Yield of cheese ^a =86.5 Schreiber melt ^b =4.8	
^a Per cent yield of cheese based on quantities of ingredients weighed and the actual cheese quantity obtained, ^b =Arbitrary value. ($n=6$)	(c) 82 °C	Slight browning on pizza. % Yield of cheese ^a =85.0 Schreiber melt ^b =4.6 More browning on pizza.	

led to decrease in meltability (Table 3). Hence processing temperature of 80 $^{\circ}$ C was selected.

The formulation arrived at and the process standardized in the present investigation for manufacture of MCA based on rennet casein and plastic cream is recommended for preparing an acceptable quality product, possessing all the characteristics required for pizza application.

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