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Research Article

Influence of Screw Speed on Texture, Colour and Microstructure of String Cheese

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Abstract: An understanding of factors that affect cheese microstructure is important, as this microstructure influences cheese physical properties. The aim of this study was to investigate the influence of screw speed on texture, colour, fat particle size and microstructure of cheese samples during the manufacture of string cheese. Moisture, protein and fat decreased and pH value increased after curds were stretched. The SS40 samples had consistently highest hardness, cohesiveness gumminess, chewiness and resilience values. The fat particle size of string cheese was significantly influenced by the screw speed. Stretched cheeses showed lower L*, a*, b* and C values than control cheese. Scanning electron microscopy of string cheese indicated that cheese microstructure changed from amorphous three-dimensional structure into a near-linear parallel fibrous structure after stretching. Results from this study indicate that the stretching treatment does decrease expressible serum and results in a more firmer protein matrix.

Keywords: Colour, fat particle size, microstructure, screw speed, string cheese, texture

INTRODUCTION

String cheese is a pasta filata cheese and a fresh cheese that can be eaten the day after it is manufactured. String cheese was produced with acute stretching conditions than Mozzarella cheese manufactured. Therefore string cheese has light taste, higher security and shorter production cycles.

More recently it has been recognized in processed cheese products that physical properties are influenced by processing conditions. However, the influence of processing conditions on natural cheese products has usually been treated empirically by manufacturers and has been analyzed on limited types of processing conditions, which may produce biased conclusions (Ma *et al*., 2013). For mozzarella, it has been reported that its properties are affected by the production processes, including milk composition, manufacturing conditions and post-manufacturing conditions (Lee *et al*., 2004).

The pasta flata process of cooking and stretching of cheese curd is typical during manufacture of string cheese. This process imparts unique characteristics to string cheese. Mechanical mixers with single screw or twin screws, coupled with steam injection system, are used for heating and stretching of string cheese. In a broad sense, this cooker-strectcher operation is an extrusion process. Although the cheese microstructure formation begins during coagulation (Oberg *et al*., 1993), it is during kneading and stretching of curd that the typical microstructure of pasta flata cheeses is established because of continuous interactions of proteins by calcium crossing bonds (McMahon *et al*., 1999). While functional properties of cheese are determined by its microstructure, study of this physical property has become important.

Some research has been published on the impact of pasta filata processing parameters on functional properties of Mozzarella cheese. Renda *et al*. (1997) determined the impact of screw speeds of the mixer at low temperature on chemical composition, proteolysis and free-oil release of low moisture part-skim Mozzarella cheese during storage. Kindstedt *et al*. (1995) investigated the influence of screw speed and residence time at high stretching temperature on composition, proteolysis, meltability and the water phase of Mozzarella cheese during storage. Kindstedt *et al*. (1995) found the combination of high stretching water temperature and slow screw speed resulted in slower proteolysis and slower meltability changes during aging. Yu and Gunasekaran (2005) analyzed the compositional, free-oil release and shear modulus of cheeses manufactured under different conditions and

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relationships between system variables and cheese shear modulus and compositional properties were established. It is well known that cheese texture, determined by chemical composition and physical properties, is largely a function of cheese microstructure. The aim of this study was to investigate the influence of screw speed on texture, colour, fat particle size and microstructure of cheese samples during the manufacture of string cheese, providing a theoretical basis for manufacturing string cheese.

MATERIALS AND METHODS

Cheese making: The string cheeses were made using a modification of the procedure (Yun *et al*., 1993). 40 L of cheese-milk yielded 13 to 15% string cheese. $CaCl_2$ (0.1 g/L) was added after the starter culture (TCC-3, CHR Hansen Inc, Denmark) was added. Until the milk pH reached 6.40, 0.1 g/L of chymosin (Stamix1150, CHR Hansen Inc, Denmark) was added. The whey was drained until whey pH reached 6.00. The curd was milled until curd pH dropped to 5.40. The salted curds were divided into four portions. The treatments included no stretching (Control), stretching with 40 r/min screw speeds (SS40), stretching with 45 r/min screw speeds (SS45) and stretching with 50 r/min screw speeds (SS50). A pilot-scale, open-channel, single-screw stretcher-cooker was used to plasticize, knead and extrude the curd. A temperature control system, using circulating hot water, was used to maintain a preset constant barrel temperature during operation. The plasticized curd was extruded directly from cylindrical molds attached to the stretcher-cooker. The Barrel Temperature (BT) used was 90°C. The exit temperatures of the cheese were 72.8, 67.8 and 63.9°C, respectively. The stretcher-cooker was emptied and cleaned between treatments. Stretching was performed for three independent batches of salted curds each. All samples were cooled in ice water for 1h and vacuum-packaged and stored at 4°C.

Composition and pH measurement: The composition of sample was determined 1 day after the cheese was made. The sample was grinded to obtain particles 2-3 mm in diameter. Moisture content was determined in triplicate using the forced-air oven method (AOAC International, 2000a). Total fat levels were measured in duplicate using a modification of the Babcock procedure (Kosikowski and Mistry, 1997). Total nitrogen was measured in triplicate using a UDK-142 nitrogen analyzer (Velp Scientific, Italy). Ash content was obtained in duplicate using the furnace method (AOAC International, 2000b). A pH meter was used to measure pH directly on grinded samples.

Expressible serum measurement: Cheeses were evaluated for expressible serum 1 day after the cheese was made. The level of Serum Expressed (ES) from grated cheese (15 g), on centrifugation at 12500 r/min for 60 min at 25°C, was used as an index of the water holding capacity of the casein matrix (Kuo *et al*., 2001). The ES was weighed and expressed as a percentage of sample mass.

Texture and rheological characteristics: At 1 day after manufacture, one block of cheese from each treatment was removed from 4°C storage and divided for testing. Samples for texture and rheological properties analysis were warmed to room temperature for 1 h prior to testing.

Texture profile analysis: Textural properties were obtained as described by Adhikari *et al*. (2003). The texture profile was determined allowing the probe to compress the sample at a speed of 1 mm/s. The compression was interrupted when the sample was compressed to 40% of their original height and the probe returned to its original position. This was followed by a 2nd compression cycle, the probe again compressing the sample at a speed of 1mm/s, the compression again being interrupted when the sample was compressed to 40% of their original height and the probe returned to its original position. The data for force as a function of time were obtained for the 2 compression-decompression cycles and using the "texture profile" function (TPA) of TA-XT2 (Stable Micro Systems, Godalming, Syrrey, UK), values for the following parameters were obtained: hardness, cohesiveness, gumminess, springiness, resilience and chewiness. All determinations were carried out in quadruplicate.

Dynamic rheology test: Rheological properties of string cheese samples were measured by dynamic Small Amplitude Oscillatory Rheology (SAOR). Triplicate discs (20 mm diameter and 1 mm in thickness) were obtained from each treatment sample and placed in a rotational rheometer (Model HAAKE MARS Ⅲ; Thermo Electron Corp., Germany). All samples were attached to the lower plate using a small amount of cyano-acrylate glue. Mineral oil was used around the periphery of the sample to prevent moisture loss. Changes in the viscoelastic properties of cheese during heating from 25 to 80°C were measured using a rotational rheometer (Lucey *et al*., 2005). All samples were heated at 5°C/min. A frequency of 0.04 Hz and a strain of 0.1% were applied to measure the storage modulus (G′), the loss modulus (G″) and phase angle [$\delta = \tan^{-1}\left(G''/G'\right)$] during heating. Phase angle indicates the arctangent ratio of viscous to elastic properties and is related to the relaxation of bonds in the matrix (Lucey, 2002), which can be used as an index of meltability of cheese (Lucey *et al*., 2003).

Evaluation of fat particle size: A sample of string cheese (0.5 g) was dispersed in a solution (50 mL) containing EDTA (0.375% w/w) and Tween 20 (0.125% v/v) and the pH was adjusted to 10 using 1 mol/L sodium hydroxide (Walstra, 1965). After standing overnight in a refrigerator (4°C), the samples were allowed to equilibrate to room temperature for 1 h. The resulting suspensions were then read in a Beckman Coulter, model LS13 320 laser diffraction particle size analyzer (Beckman Coulter Ltd., USA) (Christian *et al*., 2012). The particle size obtained, D (3, 2), was the average particle diameter, calculated as the ratio between their volume and total surface area (Chen and Liu 2012). The distribution of the fat particle sizes was also obtained and used to compare the range of particle sizes. Each sample was measured in duplicate.

Colour measurement: A CM-5 spectrocolorimeter (Minolta Camera Co., Osaka, Japan) was used to measure the colour of the cheese samples. Illuminant D65 (standard daylight) with 10° observer angle was used. Results were reported as L^*, b^* and a^* parameters, according to the CIE-LAB colour space. L^* represents lightness, $-a^*$ to a^* indicates green to red and $-b^*$ to b^* indicates blue to yellow. After the L^*, b^* and a^* parameters were read, the samples was rotated 90° and read again. This procedure was carried out in quadruplicate. Colour saturation of chroma (C value) accounts for the vividness or the colour purity from the distance between the dot (a^*, b^*) and the origin. It was calculated as $(a^{*2} + b^{*2})^{1/2}$ (Picon *et al*., 2013). The longer the distance, the more vivid or saturated a colour.

Microstructure: Scanning Electron Microscopy (SEM) was used to examine the changes in microstructure of the control and the string cheese stretched with three different screw speeds. Cheese samples were prepared following the procedures of Maria (Maria De Los *et al*., 2012). The samples were examined in a scanning electron microscope (Model SU1510; Hitachi Corp., Japan) operated at an accelerating voltage of 15 kV.

Statistical analysis: Statistical analyses of results were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC). Significant differences ($p<0.05$) among means were compared using the Bonferroni correction within the PROC MIXED program.

RESULTS AND DISCUSSION

Composition analysis: The content of moisture, fat, protein, ash and pH of the control and the string cheese stretched with three different screw speeds are shown in Table 1. Increase in screw speed resulted in a reduction in the content of moisture. The control sample that was not stretched had a moisture content of 53.81±1.46%, which was significantly higher ($p<0.05$) than the three different stretched cheese samples. The control sample that was not stretched had a protein content of 26.45±0.28%, which was significantly higher ($p<0.05$) than the SS40 sample. There were no significant in the contents of fat and ash of the control and the stretched cheese samples. The control sample had a pH value 5.08±0.03, which was significantly lower ($p<0.05$) than the stretched cheese samples. This observation suggested starter culture and chymosin activities of the control were stronger than the stretched samples. The highest mixer screw speed produced cheeses with a lower moisture content and higher protein content.

Expressible serum concentration analysis: All samples were centrifuged to obtain expressible serum (Table 2). The amount of expressible serum provides an index of the water-holding properties of the cheese. Similar to previews results (Maria De Los *et al*., 2012), the level of ES decreased in all cheeses indicating an increase in the water holding of the casein matrix. Upon light centrifugation, the amount of expressible serum varied with the stretching treatment. The amount of expressible serum of cheese decreased in the following sequence: Control>SS40>SS50>SS45. The control sample, which would have no disruption to the protein matrix, had the highest amount of expressible serum, whereas the SS45 samples had the lowest amount of expressible serum. The control sample had a expressible serum amount of 21.41±0.28%, which was significantly higher ($p<0.05$) than the stretched cheese samples. The SS40 sample had a expressible serum amount of 16.45±1.02%, which was significantly higher ($p<0.05$) than the SS45 sample. There were no

Table 1: Compositional and pH means for the control and the string cheese stretched with three different screw speeds

Samples	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	pH
Control	$53.81±1.46^{a}$	$22.30±1.04^{a}$	$26.45±0.28^{a}$	$2.84±0.04^{a}$	$5.08±0.03^{b}$
SS40	$47.78±1.30^{b}$	$21.7±2.27^{a}$	$24.84±0.35^{b}$	$2.81±0.04^{a}$	$5.33±0.02^{a}$
SS45	$45.30±1.57^{b}$	$19.1±1.28^{a}$	$25.72±0.32^{ab}$	$2.73±0.06^{a}$	$5.30±0.03^{a}$
SS50	$45.02±0.73^{b}$	$22.25±2.62^{a}$	$26.14±0.04^{a}$	$2.79±0.06^{a}$	$5.23±0.02^{a}$

$^{a-c}$: Means with the same letter within a column are not significantly different ($p<0.05$)

Table 2: Expressible serum means for the control and the string cheese stretched with three different screw speeds

	Control	SS40	SS45	SS50
Expressible serum (%)	$21.41±0.28^{a}$	$16.45±1.02^{b}$	$11.40±1.80^{c}$	$14.85±0.07^{bc}$

$^{a-c}$: Means with the same letter in the same line are not significantly different ($p<0.05$)

Table 3: Texture properties for the control and the string cheese stretched with three different screw speeds

Samples	Hardness (g)	Cohesiveness	Chewiness	Gumminess	Springiness	Resilience
Control	553.7±48.4[c]	0.75±0.02[b]	390.5±41.4[c]	417.2±42.7[c]	0.94±0.04[a]	0.33±0.02[c]
SS40	1937.1±32.5[a]	0.84±0.01[a]	1525.3±37.6[a]	1618.1±32.3[a]	0.94±0.01[a]	0.49±0.0[a]
SS45	1657.2±70.0[b]	0.78±0.03[ab]	1197.6±125.9[b]	1288.4±106.3[b]	0.93±0.03[a]	0.42±0.0[b]
SS50	614.7±49.4[c]	0.74±0.04[b]	440.7±57.9[c]	456.8±45.4[c]	0.96±0.04[a]	0.36±0.03[c]

[a-c]: Means with the same letter within a column are not significantly different ($p<0.05$)

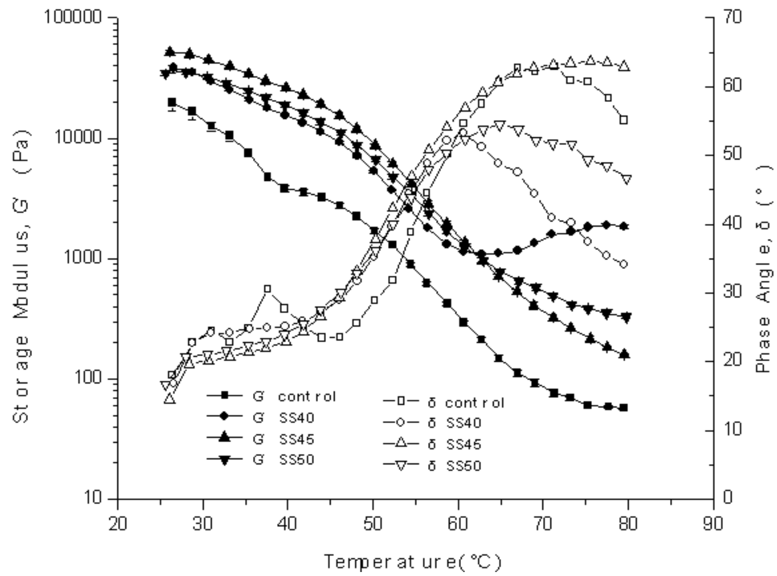

Fig. 1: Temperature-storage modulus and phase angle sweep of the control and the string cheese stretched with three different screw speeds

significant in the amount of expressible serum of the SS50 sample and the SS40 sample or the SS45 sample. The results showed the amount of expressible serum was significantly affected by the stretching treatment and the SS45 sample had the highest water holding of the casein matrix.

Texture profile analysis: The texture properties of the control and the string cheese stretched with three different screw speeds are presented in Table 3. Hardness, gumminess, chewiness and resilience of the samples decreased in the following sequence: SS40>SS45>SS50>Control. There were significant difference in hardness, gumminess, chewiness and resilience of three stretched cheese. There were no significant difference in hardness, gumminess, chewiness and resilience of the SS50 and the control. No significant differences in springiness values were recorded among all samples. The SS40 samples had consistently highest hardness, cohesiveness gumminess, chewiness and resilience. Some researchers reported that the slowest screw speed caused a slower rate of breakdown of the cheese. Some researchers also reported that cheese made with the slowest screw speed, which stayed for the longest time in the stretcher, had the most white whey loss, so that its texture properties were the highest value.

Dynamic rheology: Changes in viscoelasticity and phase angle (δ) as a function of temperature are presented for the control and the string cheese stretched with three different screw speeds in Fig. 1. The storage modulus (G′) of stretched cheese samples was higher than the control. Rheological data showed that the stretched samples were firmer than the control. The stretched cheeses flows when heated but retains some structural integrity because of the alignment of the casein fibers, which traps melted fat in columns (Guinee *et al*., 1999). The viscoelastic properties, which relate to the strength of the component interaction or bonds within the matrix, supported the fact that the string cheese was a semi-hard cheese with the stronger microstructure. For all samples, heating resulted in a decrease in G′. The G′ is a measure of the energy stored per oscillation cycle, which can be used as the index of stiffness or elastic character and when it decreases, it indicates the softening of cheese.

The phase angle of the control sample had a small peak in 37.6°C, caused by the melted fat and the phase angle of other samples had no significant peak shape in 37.6°C. This phenomenon indicated that the control sample and other stretched samples differed in fat crystallization and in microstructure of protein network. For all samples, heating resulted in a decrease in G′ and an increase in δ in the temperature region from 25 to 60°C. The changes in G′ and δ, in all samples, indicated a transition from unheated cheese, which is largely elastic in nature (δ~14-18° at 25°C), to a melted cheese which is more viscous in nature (δ ~ 52-57° at 60°C).

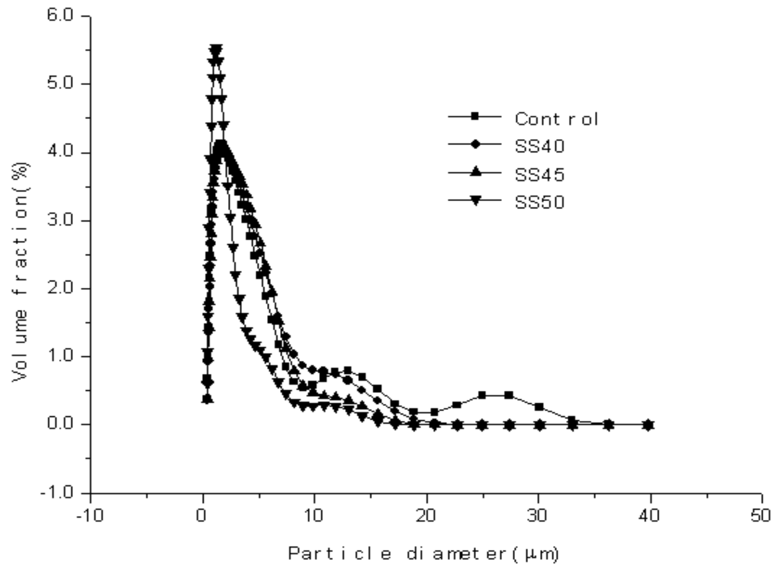

Fig. 2: Fat particle size distributions in the control and the string cheese stretched with three different screw speeds

In the temperature region from 60 to 80°C, the storage modulus (G′) of the stretched samples decreased in the following sequence: SS40>SS50>SS45. The maximum in δ was observed between 60 and 76°C for all samples. The SS45 sample had maximum δ (63.66°) and the highest temperature (75.55°C) at maximum δ, suggesting an increased fluidity of the melted cheese (Edvard, 2008). Once the δ_{max} were obtained, the δ values steadily decreased (Fig. 1).

It has been previously reported that there is a correlation between the viscoelastic parameters and flowability, with low value of δ_{max} and high values of G'_{min} coinciding with low flowability (Guinee *et al*., 1999). The SS40 sample had the lowest value of δ_{max} (53.20°) and the highest values of G'_{min} (Fig. 1), suggesting the lowest flow ability. The highest exit temperature of the SS40 sample resulted in excessive loss of white whey. The excessive loss of white whey led to the lowest flow ability of the SS40 sample. The 50 r/min screw speed reduces the size of the fat droplet and embeds casein micells around the surface of droplet, thus restricting the ability of the cheese matrix to flow when heated (Van Hekken *et al*., 2012).

Fat particle size: Figure 2 shows fat particle size distributions in the control and the string cheese stretched with three different screw speeds. It can be seen that cheese stretched at 50 r/min presented a narrow range of particles size and a more homogeneous distribution, while the others presented more wide distribution.

Mean values for the fat particle sizes of the control sample and the string cheese samples stretched at 40 r/min, 45 r/min and 50 r/min were 3.324, 3.023, 2.770 and 1.925 μm, respectively. The fat particle size of all samples were influenced by screw speed significantly ($p<0.05$), string cheese stretched at 50 r/min presenting the smallest and homogeneous fat particles size than the others, which is consistent with the results observed in the Fig. 3d. This observation suggested fat globules were sheared smaller with the increasing screw speed. While the curd is kneaded and stretched, proteins continue to interact until an incompatible compound such as fat hampers this interact. As a result, among the protein fibres, cavities or columns (fat-whey channels) are formed where the fat and water phase are retained along with any bacteria present (Joshi *et al*., 2004). Therefore, fat has an important function establishment of pasta filata cheese microstructure. Small fat particle size and an increase in size homogeneity enhance the incorporation of fat into the matrix.

Colour analysis: The analysis of variance revealed a significant ($p<0.05$) effect of treatment on all colour characteristics (Table 4). Lower L* (lightness) values were recorded for cheeses stretched at 40 r/min or 45 r/min than for cheeses stretched at 50 r/min or the control and the stretched cheese showed lower values than the control curd. Differences in matter content could explain this observation. This result has been related to fat content of cheese.

The a* values of all samples were in the greenish direction, whereas the b* values were in the yellow direction. The stretched cheese showed lower a*, b* or C than the control. For the a* values of stretched cheese, the higher screw speed, the higher a* values. No clear trend could be established for b* or C values of stretched cheese. The lowest b* or C value was recorded for cheese stretched at 45 r/min. With respect to the b* value, no significant differences were recorded among all samples. No significant differences in C values were recorded among all samples.

Table 4: Mean values for the colour parameters L*, b*, a* and C for the control and the string cheese stretched with three different screw speeds

	Control	SS40	SS45	SS50
a*	-1.20±0.12[a]	-2.64±0.26[b]	-2.59±0.26[b]	-2.39±0.06[b]
b*	11.84±1.22[a]	11.09±0.26[a]	10.38±0.52[a]	10.98±0.64[a]
L*	83.95±1.73[a]	77.84±1.84[b]	76.40±1.37[b]	80.36±1.86[ab]
C	11.90±1.22[a]	11.40±0.30[a]	10.70±0.56[a]	11.24±0.61[a]

[a-c]: Means with the same letter in the same line are not significantly different ($p<0.05$)

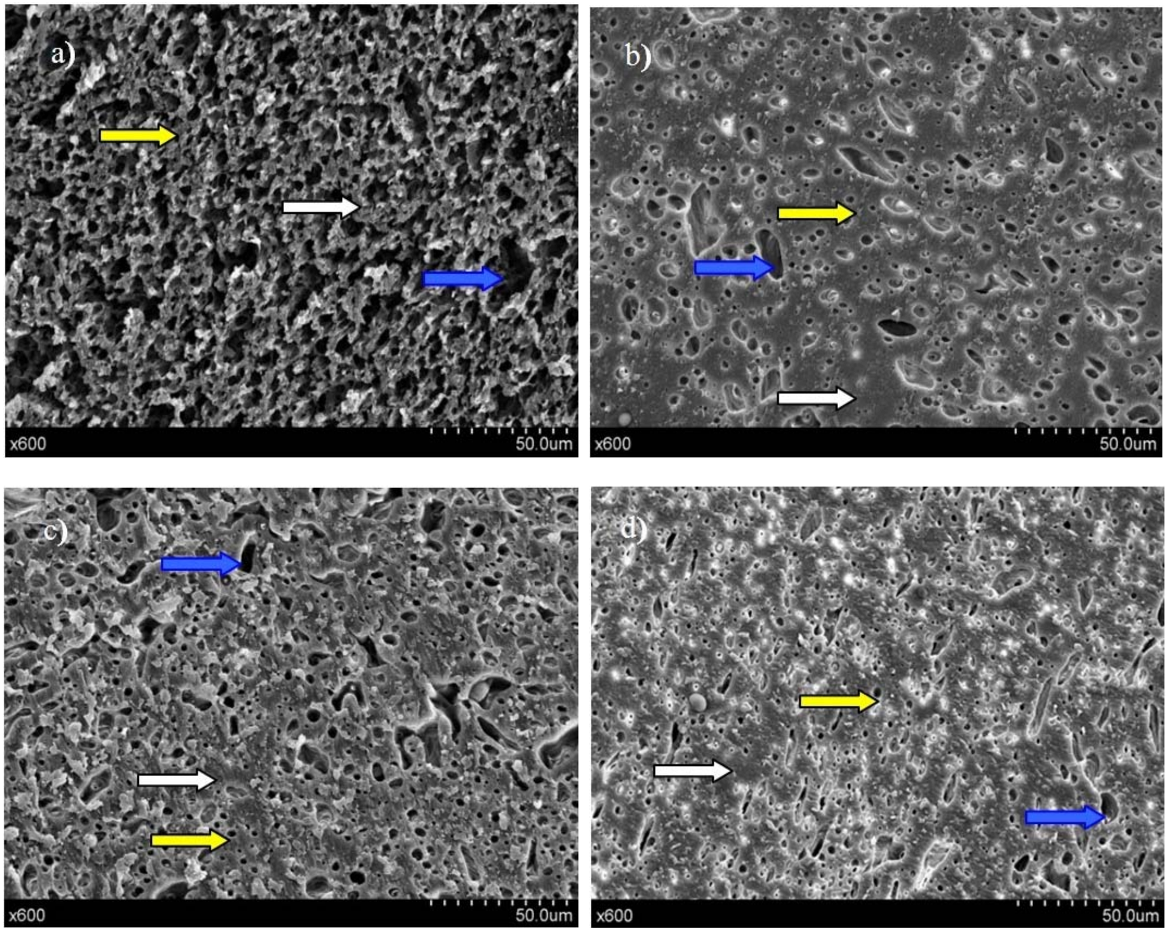

Fig. 3: Scanning electron micrographs of the cross sections of the control and the string cheese stretched with three different screw speeds; a): control; b): stretching at 40 r/min; c): stretching at 45 r/min; d): stretching at 50 r/min; white arrow refers smooth protein matrix; Blue arrow refers water cavities. Yellow arrow refers spherical spots of fat globules

Microstructure: To a great extent, the changes in texture, rheological properties of cheese are caused by changes in cheese microstructure, therefore it is important to study how stretching treatments influence the cheese microstructure. The SEM micrographs of the control and the string cheese stretched with three different screw speeds are shown in Fig. 3 and 4. The screw speed was 40, 45 and 50 r/min at barrel temperature 90°C. The exit temperatures of the cheese were 72.8, 67.8 and 63.9°C for BT 90°C. It is clearly seen that the microstructure of the cheeses depended on screw speed and barrel temperature parameters. The control sample that was not stretched had loose amorphous three-dimensional structure of the protein (Fig. 3a). Cheese was heated and stretched, causing the casein fibers to be elongated and aligned (Tunick, 2010), therefore the three-dimensional network structure of the protein strands can be observed (Fig 3b to d and 4). The stretched cheese showed continuous protein matrix decorated by irregular holes (which represent free-water) and spherical spots (which represent fat globules) (Fig. 3b to d). The SS40 sample had more the area occupied by smooth of casein matrix (Fig. 3b). A less rate of breakdown of the casein matrix and excessive loss of white whey in the protein matrix at higher stretching temperature explained this observation. This result is consistent with the changes in hardness, cohesiveness gumminess, chewiness and resilience. It suggested cheese texture was strongly influenced by cheese microstructure. As the screw speed increased from 40 r/min to 50 r/min, the microstructure showed a decrease in size and number of

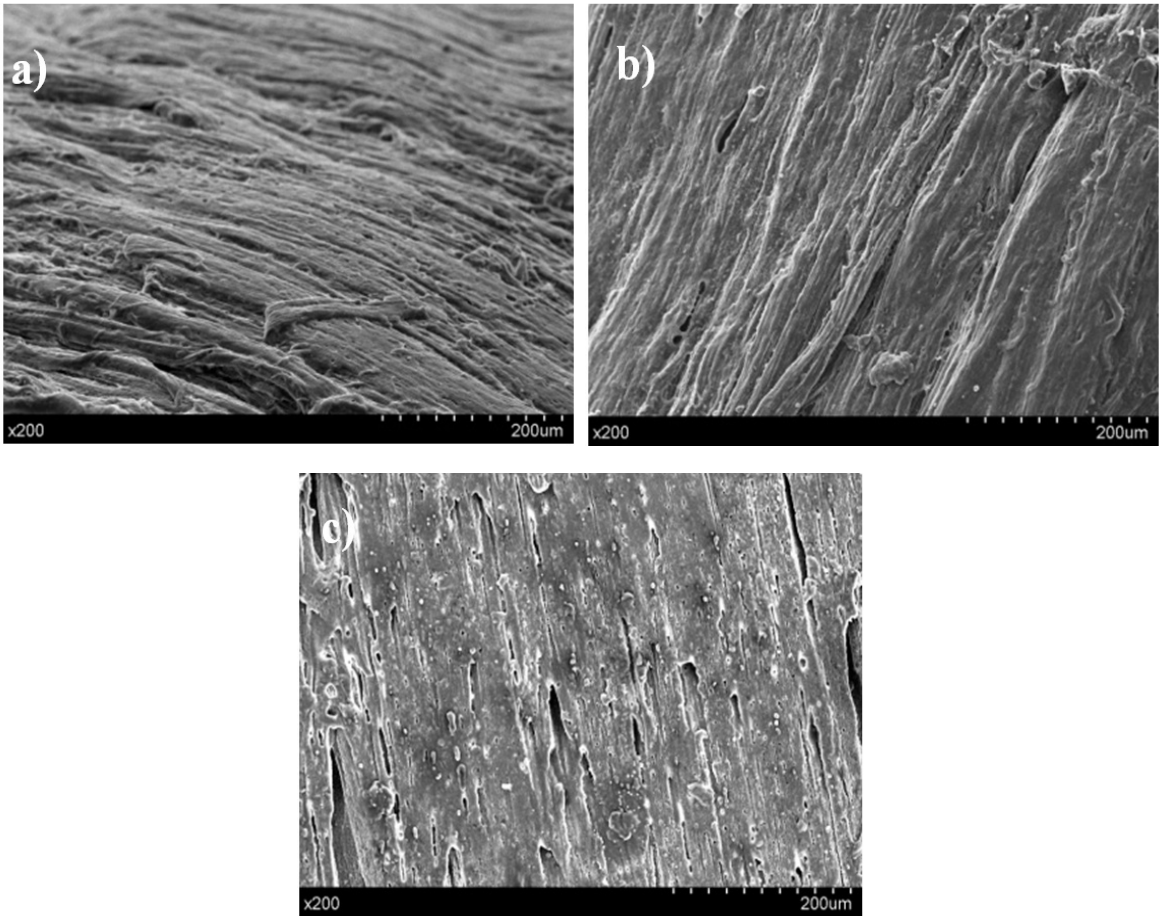

Fig. 4: Scanning electron micrographs of the longitudinal sections of the string cheese; a): stretching at 40 r/min; b): stretching at 45 r/min; c): stretching at 50 r/min

water cavities and a concomitant increase in the number of and a reduction in the size of fat globules, consistent with the changes in fat particle size distributions (Fig. 2). Also, the fat globules were distributed more uniformly.

The wider protein strands were observed in the micrograph of cheese stretched at 50 r/min (Fig. 4c). Cheese stretched at 50 r/min presented the smallest fat particles size. Small fat particle size enhanced the incorporation of fat into the matrix (Fig. 3d), which may caused reducing of fat phase among the protein strand and a consequent coalescence in the protein fibres.

CONCLUSION

The present study suggests that cooking and stretching treatments during pasta filata process have a significant influence on the moisture and pH value of the cheese. Stretching resulted in string cheese that was less expressible serum. The SS40 samples had consistently highest hardness, cohesiveness gumminess, chewiness and resilience. The storage modulus (G′) of stretched cheese samples was higher than the control. The fat particle size of string cheese was significantly influenced by the screw speed. Stretched cheeses showed lower L*, a*, b* and C values than control cheese, although no significant differences in b* and C values were recorded among cheeses. In addition, results from this study indicate that the stretching treatment results in a more firmer protein matrix. Cheese manufactures can use this information to produce string cheese that meets the demands of their users.

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