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Effect of Starter Culture Types on Textural, Rheological, and Melting Properties of Spreadable Processed Cheese Made from UF Milk Retentate

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STARTER cultures types have a substantial effect on the structural characteristics of spreadable processed cheese made from Ultra filtered (UF) milk retentate and can minimize the final product cost. In this study, the effect of starter culture type which had been used on the meltability, firmness and rheological properties of the processed spreadable cheese manufactured from UF milk retentate was investigated. Standardized UF processed cheese was inoculated with three starter cultures (ABT-8, CHN-22 and FRC-60). Half of the inoculated starter cultures samples were incubated at 25° C for 24 hr while the other half was incubated at 25 °C for 72 hr. The control treatment was prepared without adding any starter cultures. Rheological modelling, firmness and meltability of UF processed cheese properties were examined through the period of 3 months of storage at 6±2 °C. Significant differences in flow behaviour indexes were shown as a result of adding various starter cultures during storage and PC22-3 had the highest value. The highest decrease in consistency coefficient (*k*-values) was found with PC8-1 and PC60-1 throughout the storage period. Viscosity of processed cheese decreased corresponding to the increase in shear rate in all treatments. Furthermore, all the tested samples displayed the typical Herschel-Bulkley model having shear-thinning Pseudoplastic, while the flow curves lacked a linear characteristic. The cheese structure was rapidly broken down after the initial shearing. However, the breakdown rate decreased at the higher shear rate. Additionally, the treatments fermented with starter cultures (PC8-1, PC22-1 and PC60-1) for 24 hr had lower firmness than control and other treatments. Good meltability for all starter cultures treatments was obtained corresponding to the two factors, fermentation time and storage period.

Keywords: UF milk retentate, Spreadable processed cheese, Starter cultures, Rheological properties, Meltability.

Introduction

Processed cheese has become very popular and is consumed by many people in different countries, which led to a significant increase in its production (Fu and Nakamura, 2018). Processed cheeses are a major food product that appears in a variety of forms including spreads, slices and blocks and are used as raw material for further processing in cheese industry (Solowiej et al., 2010; Černíková et al., 2017). Spreadable processed cheese is described according to Codex Alimentarius as a dairy product made from one or more types of cheese in different stages of ripening with or

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without the addition of milk ingredients or other foodstuffs by grinding, mixing, melting and emulsifying in the presence of emulsifying salts and cooking at high temperature (Černíková et al., 2017). It has always been traditionally prepared using a mixture of several natural cheeses along with a variety of dairy products like buttermilk, butter, cream, anhydrous butterfat, whey protein concentrate, milk powder, skimmed milk powder, etc., along with non-dairy products like preservatives, stabilizers, spices, vegetable oils, mushrooms, flavour enhancers, etc. Emulsifying salts are considered an important food ingredient in the production of processed cheese (the most common types are sodium salts of phosphates, polyphosphates, citrates, or their mixtures). They are used for emulsifying fat with the casein proteins, hydrating the free water, and obtained homogeneity in the end product (Nagyová et al., 2014). The emulsification process improves the consistency of the processed cheese, in particular its viscosity and mechanical properties (Fu and Nakamura, 2018).

Determining the rheological characteristics of any cheese, especially the processed cheese, is important, as it describes the general changes in texture that occur during the cheese processing. It also helps in determining the cheese structure based on their composition, storage conditions and processing methods (Kapoor & Metzger, 2008 and Solowiej, 2012). Processed cheese texture is affected by numerous factors, like the type and character of the natural cheeses, quality of raw cheese used, emulsifying salts, storage, pH, moisture and fat content, pre-cooked cheeses, processing duration, addition of non-dairy or dairy products (Buňka et al., 2012; Guinee & O'Callaghan, 2013 and Fu et al., 2018). New applications, focusing on processed cheese analogues or imitations made with partial or complete substitution of natural cheeses with milk proteins or other protein ingredients, are being currently developed (Solowiej et al., 2010 and El-Garhi et al., 2018).

The processed cheese was produced using the UF milk retentate as a partial substitute of the natural cheese. The ultrafiltration process helps in concentrating the milk before forming or handling the curd and prevents whey removal during the cheese manufacturing process (Mistry and Maubois, 2017). The UF process increases the protein, fat and moisture content in the cheese, in comparison to the cheeses that are manufactured using the conventional techniques. The UF cheeses show a higher concentration of whey proteins and display a higher water retention capacity (Karami et al., 2009). The UF technique showed many advantages like reducing the starter culture concentration, energy, rennet and time required for cheese manufacturing (Benfeldt, 2006). In general, processed cheese made from UF milk retentate is grainy, hard, crumbly, sticky and bland, compared to conventional processed cheese made by mixing various types of natural cheeses with varying degrees of maturation (Sharma et al., 1989). Use of starter culture is a convenient technical process which helps in improving the texture, flavour, appearance and functionality of cheese made with the UF milk retentate.

Starter mesophilic and thermophilic cultures or their mixture are important starter cultures used in the development of many fermented dairy products (Alegría et al., 2016). During the cheese ripening process, casein proteolysis forms the basis for the production of a large variety of amino acids and peptides, for improving the cheese flavour, texture and taste (de Azambuja et al., 2017). During the fermentation process, the main function of starter cultures is to produce lactic acid in sufficient amounts, which plays a significant role in the biological preservation of products. They also increase food safety as they produce many important antimicrobial products, like H_2O_2, organic acids and bacteriocins (Topisirovic et al., 2006). Few systematic studies have been carried out on the use of UF milk retentate in processed cheese manufacture as a whole substitute of natural cheeses. A few studies determined the relationship between the rheological characteristics of the UF processed cheese and the starter culture type, with regards to the cheese texture. Therefore, the purpose of the present study is to investigate the improvement of the texture, meltability and rheological characteristics of processed cheese through its manufacturing from UF milk retentate with different types of starter cultures, fermentation time and storage periods.

Materials and Methods

Materials

Bovine raw milk (4.2% fat) was obtained from Faculty of Agriculture farm, Cairo University (Giza, Egypt). Milk Protein Concentrate (MPC-70) powder was obtained from an Idaho company, USA. It composed of 71.8% protein, 15% lactose, 7% ash, 5% moisture and 1.2% fat. Butter was acquired from the New Zealand Trade Centre Ltd., Auckland, New Zealand. Three commercial starter cultures of Chr. Hansen's Laboratories (Copenhagen, Denmark) were used to ferment UF milk retentate. The cultures used were thermophilic ABT-8 culture (*Lactobacillus acidophilus*, *Bifidobacterium bifidus*, *Streptococcus salivarius subsp thermophilus*), mesophilic CHN-22 culture (*Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp cremoris*, *Leuconostoc citrovorum* and *Lactococcus lactis subsp lactis biovar diacetylactis*), and mesophilic & thermophilic starter culture FRC-60 (*Lactococcus lactis subsp lactis biovar diacetylactis*, *Streptococcus salivarius subsp thermophilus* and *Lactobacillus delbrukii subsp bulgaricus*. A commercial emulsifying sodium polyphosphate salt mixture (BDH, England) was used for manufacturing processed cheese. Soya lecithin and Creamocy 200 were used as fat emulsifier and stabilizer, respectively. They were supplied by MEFAD Company, Egypt. Fine grade commercial salt (NaCl) was obtained from the local market.

Methods

Preparation of UF milk retentate

Milk was heated up to 50-52 °C and then subjected to ultrafiltration (carbon membrane with a pore size of 0.05-0.1 µm). The milk was concentrated for 3-folds (Volumetric Concentration Factor, 3X VCF). UF milk retentate (Protein 10.2%, fat 18%, lactose 4.2%, ash 1.6%, and moisture 66%) was cooled after pasteurization at 75°C/15 sec.

Preparation of spreadable processed cheese

The pH of the treatments was adjusted to 5.2-5.3 before adding other components for standardising the UF milk for processed cheese manufacturing. The fat and protein concentrations were standardised to 25 and 12%, respectively, by adding butter and MPC-70 for a final ratio of fat to dry matter not less than 55%, for achieving a high quality spreadable cheese. Treatments were cooked in a cheese cooking pan for 2-3 min. at 85 °C with 2% emulsifying salt, 0.1% fat emulsifier and 1% stabilizer. Standardized cooked processed cheese was divided into four treatments; one was set as a control (without starter culture), while the other three treatments were inoculated in aseptic conditions with three commercial starter cultures (ABT-8, CHN-22 and FRC-60, respectively) and poured into plastic containers. Half of these plastic containers were incubated at 25 °C for 24 h (PC8-1, PC22-1 and PC60-1). The other half was incubated at 25 °C for 72 h (PC8-3, PC22-3 and PC60-3). The produced cheeses were stored for 3 months in a refrigerator at 6±2 °C. Temperature of the samples was adapted at 21 °C before analysis. Cheese samples were analysed periodically when fresh, 30, 60 and 90 days of storage. The analysis was done in triplicate for each sample.

Rheological measurements (Methods of apparent viscosity and firmness)

The apparent viscosity of processed cheese was measured using Rotational Physica MCR 300 Rheometer (Physica Messtechnic GmbH, Stuttgart, Germany) with parallel plate (PP25) and 0.5mm gap. After preparing the processed cheese, viscosity and shear stress were measured periodically (1, 30, 60 and 90 days) at shear rates ranging from 10 to 50 sec^{-1}. After two minutes of spindle rotation, shear stress corresponding to each shear rate was recorded to ensure steady reading. All samples were calibrated at 21±1 ºC using a Paar Physica circulating bath and a controlled peltier method (TEK200) with a precision of ±0.1 °C before loading into the rheometer device.

The rheological analysis was conducted using a software program, which assessed the spindle torque, speed, viscosity, shear rate, shear stress, and the elapsed time for the data points. All shear stress/ shear rate figures were transferred to the Microsoft Excel sheet and used for determining the Power Law and Herschel-Bulkley model equations. The Power Law materials were characterised as per the Power Law calculation, using a straight-line log-log graph of shear stress *vs*. shear rate.

$$\sigma = K\gamma^n \qquad (1)$$

Where;
- σ : Shear stress [Pas]
- K : Consistency index [Pas S^n]
- γ : Shear rate [s^{-1}]
- n : Dimensionless number indicating Newtonian flow closeness

A straight-line graph of shear stress *vs.* shear rate helps in applying the Herschel-Bulkley model, used for determining the yield stress (σ_0) in the following manner:

$$\sigma = \sigma_0 + \eta_a \gamma \qquad (2)$$

Where
- σ : Shear stress
- σ_0 : yield stress [Pas]
- η_a : apparent viscosity [Pas. s^{-1}]
- γ : Shear rate [s^{-1}]

Texture analysis

The firmness of the cheese using the cone penetrometer (Stanhope Seta, Surrey, UK) was measured at 15 ^{o}C. The penetrometer was fitted with two separate weights: a standardized 47.5 g rod weight and an additional 35 g weight, as the total used weight was 82.5 g. The entire scale was calibrated into 35 units; each unit was further divided into 10 parts, each one of 0.1 mm. The cheese sample was placed on the base for measurement and the rod moves down until the cone tip simply rests on the surface of the cheese. The cone releasing the button was then pressed 5s to measure distance in 0.1 mm units. The measurement was repeated 3 times on the surface of the cheese sample in three different locations. The average depth of penetration (in millimetres) was reported as the value of penetration reversing the value of firmness.

Meltability

UF processed cheese meltability was measured with the modified Schreiber test. The cheese samples (4.8 mm thickness and 41 mm diameter) were put in some Petri dishes and heated in the microwave oven (Samsung, South Korea) for 60s at 300 W, and then cooled. The expansion of the samples was measured along 6 lines that were marked in concentric circles (Mleko & Foegeding, 2000). The Schreiber meltability was an average of 3 values for the triplicate samples (at a random scale of 0–10 units).

Statistical analysis

The two-way analysis of variance (ANOVA) was performed by running the MSTAT-C program (Ver.2.10, Michigan state Univ., USA), package on a personal computer (Freed et al., 1991). Two Factor Randomized Complete Block Design was used to analyze meltability. Least significant difference test was performed to determine differences in means at *P* □ 0.05 (Carmer et al., 1989; Higgins, 2004). Linear regression was used to correlate rheological and meltability parameters to the type of strain culture used and storage period of spreadable processed cheese.

Results and Discussion

Rheological properties

Flow behaviour Model

The variations in shear stress caused by changing the shear rate were explored to elucidate the basic flow behaviour combination of processed cheese containing a different type of starter cultures, fermented for 1 and 3 days, and preserved in the refrigerator for three months. The processed cheese samples were subjected to an increasing shear rate under a constant strain and the relationship between the shear stress and shear rate was presented in Fig 1.

A typical Herschel-Bulkley behaviour was noted, as the best linear fitting lines of the shear stress / shear rate data for control and fermented cheese samples, crossed the Y -axis at 0 shear rate with yield stresses shown in all treatments. Higher fermentation periods (3 days) of the processed cheeses resulted in an upward change of the flow curve (structure building contributing to an increase in viscosity of the samples). The shear stress increased significantly ($P < 0.001$) in the treatments inoculated with CHN-22 mesophilic starter culture. Significant differences in shear stress were clearly noticed as a result of increasing shear rate, type of starter cultures used, fermentation and storage periods ($P < 0.001$). Not only did those factors affect shear stress significantly but also the interaction between them ($P < 0.001$).

Yield stress values

The yield stress values have been calculated by fitting the shear stress and shear rate data to equation **3**:

$$\sigma = \sigma_0 + \eta_a \gamma \qquad (3)$$

σ: Shear stress

σ_0: Yield stress = (shear stress at 0 shear rate, by extrapolation)

η_a: Apparent viscosity [Pa.s] = "high shear" viscosity or approximate viscosity at an infinite shear rate (based on the slope of the plotted line).

γ: Shear rate

The 'best fit' routine was used and the line was fitted with high regression coefficients, (Table 1) for all the samples, indicating a fitting model.

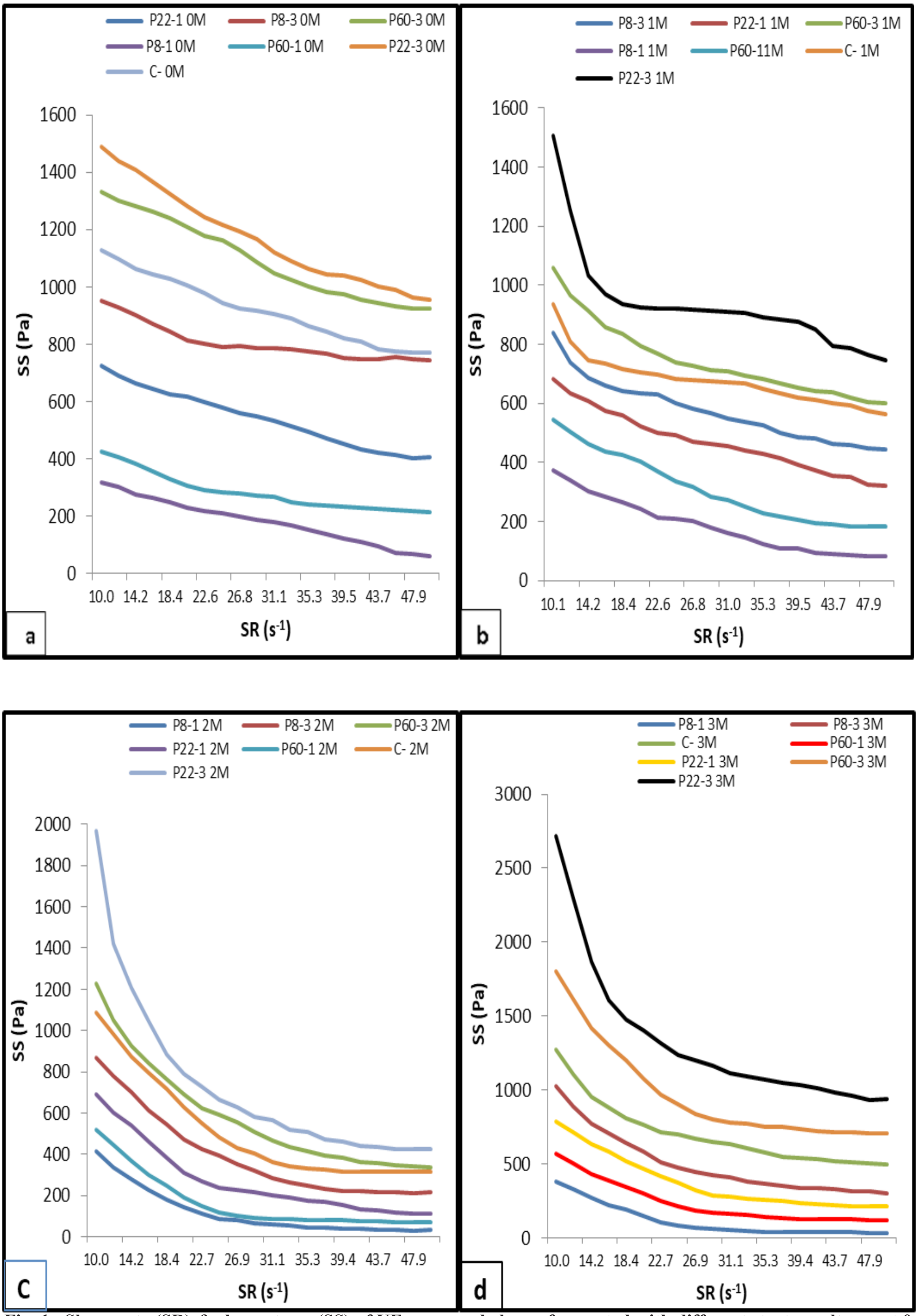

Fig. 1. Shear rate (SR) & shear stress (SS) of UF processed cheese fermented with different starter cultures at 0, 1, 2, and 3 months of cold storage (a, b, c, and d, respectively).

TABLE 1. Effect of starter cultures type, incubation time and storage periods on yield stress of UF processed cheese.

Treatments	Liner equation	Herschel-Bulkley model	
		Yield stress	R^2
	Fresh processed cheese		
Control	y = -0.0209x + 7.0342	7.034	0.991
PC22-3	y = -0.0237x + 7.3049	7.3049	0.984
PC60-3	y = -0.0214x + 7.2155	7.2155	0.9804
PC8-3	y = -0.0116x + 6.8087	6.8097	0.9439
PC22-1	y = -0.0319x + 6.6081	6.6081	0.9935
PC60-1	y = -0.0353x + 5.9952	5.9952	0.9398
PC8-1	y = -0.0829x + 5.9623	5.9623	0.9465
	Processed cheese stored for 1 month		
Control	y = -0.019x + 6.7118	6.7118	0.8822
PC22-3	y = -0.0228x + 7.067	7.067	0.7102
PC60-3	y = -0.0261x + 6.8729	6.8729	0.9277
PC8-3	y = -0.0298x + 6.6488	6.6488	0.9677
PC22-1	y = -0.0373x + 6.5161	6.5161	0.9891
PC60-1	y = -0.0631x + 6.3291	6.3291	0.9807
PC8-1	y = -0.0851x + 5.9897	5.9897	0.9879
	Processed cheese stored for 2 month		
Control	y = -0.069x + 6.8625	6.8625	0.8781
PC22-3	y = -0.0707x + 7.2263	7.2263	0.8746
PC60-3	y = -0.0667x + 6.982	6.982	0.947
PC8-3	y = -0.0782x + 6.6723	6.6723	0.9279
PC22-1	y = -0.0946x + 6.4337	6.4337	0.9547
PC60-1	y = -0.1037x + 5.9544	5.9544	0.838
PC8-1	y = -0.1377x + 5.8501	5.8501	0.9386
	Processed cheese stored for 3 month		
Control	y = -0.0441x + 6.9735	6.9735	0.9129
PC22-3	y = -0.0461x + 7.6248	7.6248	0.8299
PC60-3	y = -0.0465x + 7.3114	7.3114	0.8363
PC8-3	y = -0.0608x + 6.7728	6.7728	0.9209
PC22-1	y = -0.072x + 6.5782	6.5782	0.926
PC60-1	y = -0.0854x + 6.2071	6.2071	0.9126
PC8-1	y = -0.1347x + 5.7614	5.7614	0.8905

Control: not fermented UF processed cheese. PC22-1 and PC22-3: fermented UF processed cheese by CHN-22 starter culture for one and three days, respectively. PC60-1 and PC60-3: fermented UF processed cheese by FRC-60 starter culture for one and three days, respectively. PC8-1 and PC8-3: fermented UF processed cheese by ABT-8 starter culture for one and three days, respectively.

Herschel-Bulkley equation

Flow behaviour parameters of processed cheeses, which were influenced by the type of starter culture, fermentation and storage periods, were evaluated by fitting the shear stress/shear rate data to the Herschel-Bulkley equation (Benezech & Maingonnat, 1994);

$$\sigma - \sigma_0 = K\gamma^n \quad (4)$$

Where
: σ_0 Yield stress = (shear stress at 0 shear rate, by extrapolation)
: σ shear stress [Pas]
: K consistency index [Pa.s] = viscosity at $1s^{-1}$
: γ shear rate [s^{-1}]
: n A dimensionless number that indicates the closeness to Newtonian flow. For a Newtonian liquid n = 1; for dilatant fluid n>1; and for pseudoplastic fluid n<1

Herschel-Bulkley constants and R^2 values for double log plots for all treatments are given in Table 2. Flow behaviour index (n) gives information about shear thinning behaviour of processed cheeses. Flow behaviour index decreased with increasing storage period. Processed cheese cultured with PC8-1 had the lowest flow behaviour index throughout the storage periods which indicated a better spreadability followed by P60-1. The highest n value was possessed by the PC22-3 samples in all storage periods that indicated a strong structure and less spreadability. The flow behaviour index calculates the degree of deviation from Newtonian flow and findings were consistent with Pseudoplastic flow for which "n" is less than 1. However, the type of starter cultures significantly affected (P<0.001) the "n" value and the values varied between 0.012-0.15 and deviated from the Newtonian flow. The consistency coefficient (*k*) indicates the flow properties of processed cheese. Consistency coefficient (*k*-values) values of control cheese varied from all cheese samples. The difference in *k*-values between the samples may be referred to the variation in their acidity values. Increasing the acidity values increases the flow behaviour index and the fluid tends towards Newtonian behaviour. Similar behaviour for spreadable goat cheese was reported by Frau et al. (2014). Consistency coefficient was more affected by the type of starter culture used, fermentation and storage period than the flow behaviour index (Table 2). The plots of the consistency index (K) and flow behaviour index were shown in Fig. 2.

For the PC60-3 treatment, *k*-value was close to that of control sample for all storage periods except for the three-month-period, whereas PC22-3 showed the highest values. The decrease in *k* values was clearer in PC8-1 treatment (Ong et al., 2007) . Control sample K values insignificantly (P > 0.001) decreased during storage to reach the lowest value after 30 days, then gradually increased until the end of the storage period. During storage, *k* values of cultured samples declined during 60 days of storage and increased afterwards. This may be due to the evaporation that took place in the cheese during the storage period. Similar results were noted by Ong et al. (2007). These outcomes were in agreement with the above plot, i.e., shear-thinning line with a decreasing gradient (n < 1), wherein their inversely proportioned with increasing shear. This has been vindicated by the existence of a network structure whose bonds must be broken to allow the flow.

Apparent Viscosity

Viscosity is the resistance of the material to flow or spread (Kapoor and Metzger, 2008). Measuring the processed cheese viscosity helps in characterising the meltability of the cheese (Dimitreli & Thomareis, 2004; Sołowiej et al., 2014). Figure 3 describes the apparent viscosity values for different shear rates (ranging between 10 and 50 s^{-1}). Obtained results revealed a considerable decrease (P<0.001) in the viscosity values with increasing shear rate for all treatments with different starter cultures, fermentation and storage periods. The curves levelled off at the shear rate of $10s^{-1}$ and then decreased constantly till the maximum applied shear rate of $50s^{-1}$. This behaviour was noted for a shear-thinning system as mentioned before. Hence, the processed cheese-manufactured using different starter cultures, storage periods and fermentation conditions could be characterised by using a Herschel-Bulkley model with shear-thinning Pseudoplastic properties, wherein the flow curves showed no linear characteristic. The structure showed a rapid breakdown in initial shearing, while slow changes were noted at a higher shear rate. This behaviour was attributed to the combined effects of the breakdown of weaker linkage between the proteins or proteins and stabilisers. Reformation of these linkages could result in Brownian motion and a molecular collision.

TABLE 2. Effect of starter cultures type, incubation time and storage periods on the consistency (K) and flow behavior index (n) of UF processed cheese.

Treatments	Liner equation	Flow parameters: Consistency coefficient K (Pascal. S^2/m^2)	Flow behavior index n (-)	Power law R^2
Fresh processed cheese				
Control	y = -0.021x + 7.0291	1129.014	0.021	0.992
PC22-3	y = -0.0238x + 7.3009	1481.633	0.0238	0.9841
PC60-3	y = -0.0216x + 7.2104	1353.434	0.0216	0.9804
PC8-3	y = -0.0117x + 6.8008	898.566	0.0117	0.8441
PC22-1	y = -0.0323x + 6.5988	734.214	0.0323	0.9935
PC60-1	y = -0.0362x + 5.9793	395.164	0.0362	0.9408
PC8-1	y = -0.0874x + 5.9594	387.378	0.0874	0.9409
Processed cheese stored for 1month				
Control	y = -0.0192x + 6.7041	822.049	0.019	0.8827
PC22-3	y = -0.023x + 7.0619	1172.625	0.023	0.7107
PC60-3	y = -0.0263x + 6.866	965.745	0.026	0.9281
PC8-3	y = -0.0302x + 6.64	771.858	0.03	0.968
PC22-1	y = -0.0378x + 6.5066	675.937	0.038	0.9891
PC60-1	y = -0.0646x + 6.3201	555.573	0.065	0.9811
PC8-1	y = -0.089x + 5.9829	395.440	0.089	0.9882
Processed cheese stored for 2month				
Control	y = -0.0698x + 6.8572	943.88	0.069	0.879
PC22-3	y = -0.0712x + 7.2227	1366.49	0.071	0.876
PC60-3	y = -0.0675x + 6.977	1064.22	0.067	0.948
PC8-3	y = -0.0797x + 6.6659	788.40	0.079	0.929
PC22-1	y = -0.0975x + 6.4295	620.17	0.975	0.957
PC60-1	y = -0.1516x + 5.8703	383.75	0.108	0.843
PC8-1	y = -0.1085x + 5.9443	354.25	0.151	0.951
Processed cheese stored for 3month				
Control	y= -0.0446x + 6.9674	1064.22	0.0446	0.9136
PC22-3	y= -0.0463x + 7.6221	2038.56	0.0463	0.8303
PC60-3	y = -0.0468x + 7.3075	1495.18	0.0468	0.8367
PC8-3	y = -0.0617x + 6.7656	871.31	0.0617	0.9221
PC22-1	y = -0.0734x + 6.5712	713.37	0.0734	0.9272
PC60-1	y = -0.0882x + 6.1982	492.75	0.0882	0.9154
PC8-1	y = -0.1488x + 5.7713	320.54	0.1488	0.9028

Control: not fermented UF processed cheese. PC22-1 and PC22-3: fermented UF processed cheese by CHN-22 starter culture for one and three days, respectively. PC60-1 and PC60-3: fermented UF processed cheese by FRC-60 starter culture for one and three days, respectively. PC8-1 and PC8-3: fermented UF processed cheese by ABT-8 starter culture for one and three days, respectively.

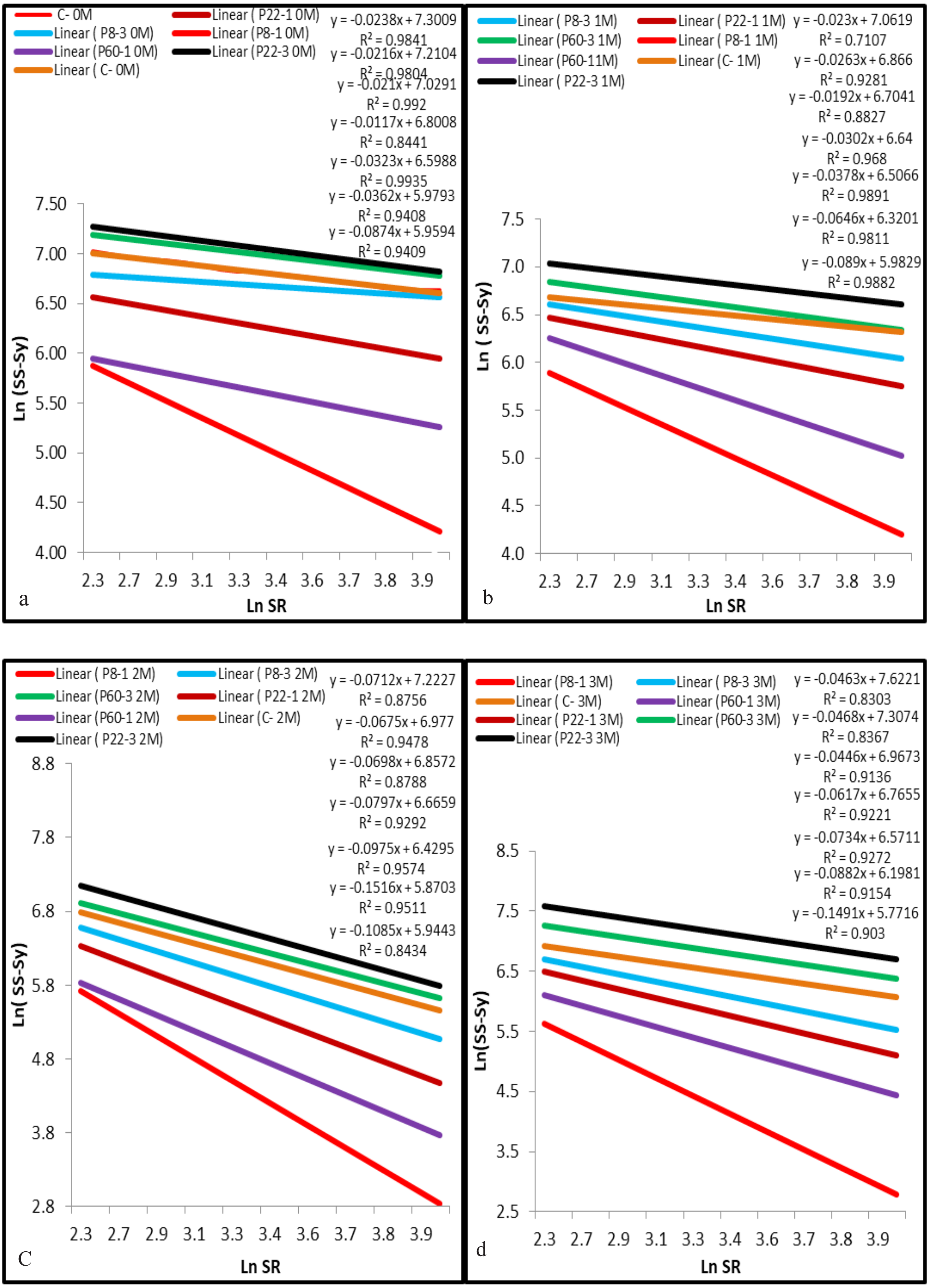

Fig. 2. Double logarithmic plot for UF processed cheese fermented with different starter cultures at 0, 1, 2, and 3 months cold storage (a, b, c, and d, respectively).

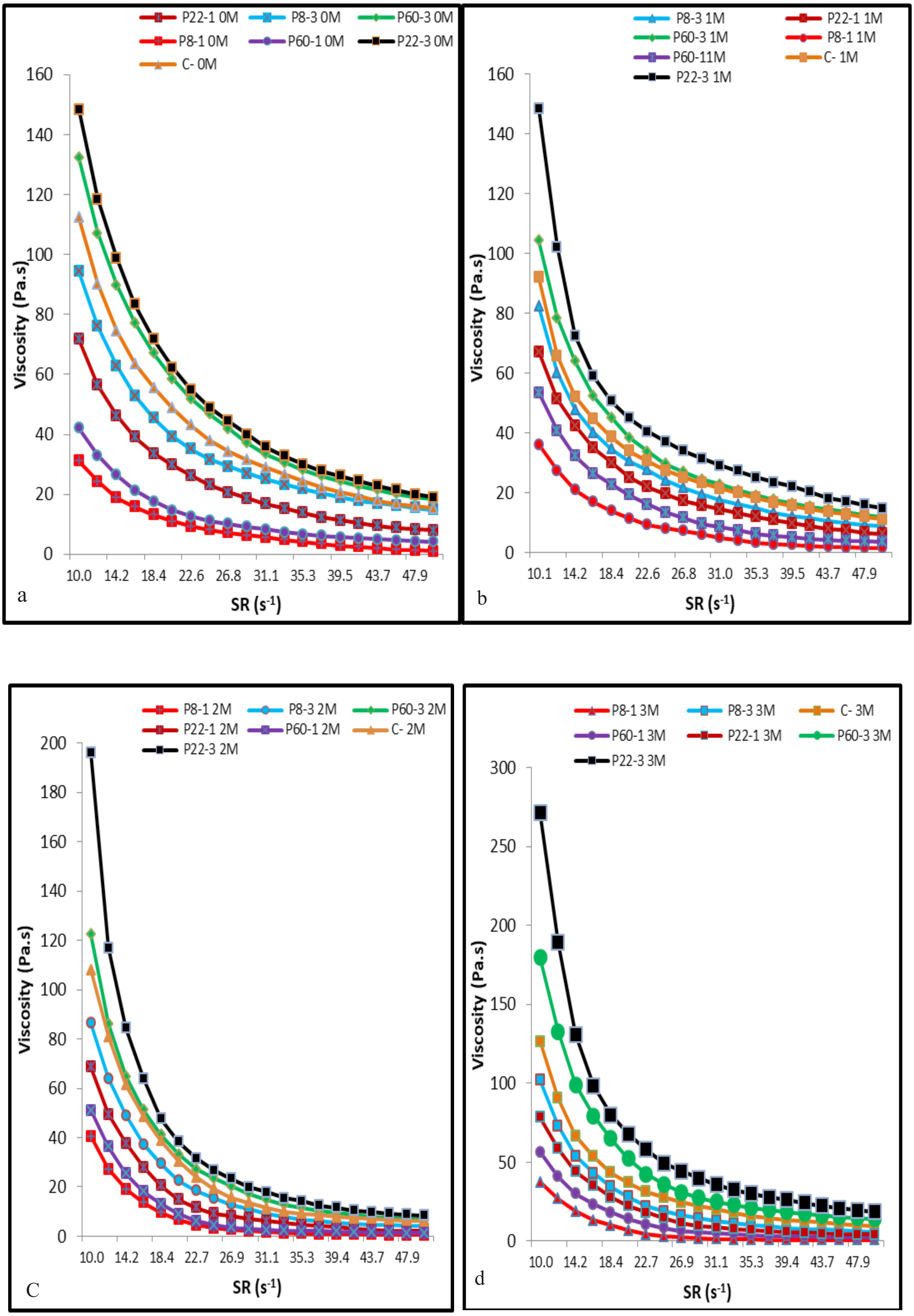

Fig. 3. Effect of shear rate on viscosity of UF processed cheese fermented with different starter cultures at 0, 1, 2, and 3 months cold storage (a, b, c, and d, respectively).

The commercial starter culture treatment PC8-1 exhibited the lowest viscosity value (31.15 Pa.s), while PC22-3 possessed the highest (148.40 Pa.s) when fresh. Moreover, treatments PC22-1 and PC60-1 exhibited high values, 71.72 and 42.4 Pa.s, at shear rate 10 s^{-1}, respectively. The treatments were viscous in descending order as follows: PC22-3 > PC60-3 > PCC > PC8-3 > PC22-1 > PC60-1 and PC8-1 The starter cultures types at different incubation time have a remarkable effect (*P*<0.001) on the viscosity of the resultant processed cheese. The cheese showed high viscosity values with increasing the incubation time. This may be due to the effect of the starter cultures on the proteolytic activity towards casein by releasing its microbial intracellular peptidases, as well as the final pH value in the processed product in the first day of incubation then built up a new structure with more firm bonds. All the above factors affected the protein network and protein association in the cheese product. Similar observations were made by Awad et al. (2004).

UF spreadable processed cheese treatments exhibited low viscosity values after one month of storage (Hesari et al., 2006) then increased along the storage period up to 3 months. The decrease in the viscosity could be attributed to protein breakdown occurred through the starter proteolytic enzyme activity (Ferrão et al., 2016) and as a result, the texture becomes softer over time (Akkerman et al., 2017).

Generally, treatments with starter cultures of PC8-3, PC22-3 and PC60-3 showed higher viscosity than that in treatments with starter cultures of PC22-1, PC8-1 and PC60-1. This could be due to the effect of fermentation time on the intensity of changes in protein degradation in final product. It seems that the commercial starter cultures can help in the improvement of body and texture in the final product.

Texture and Principal component analysis

Firmness, which was inversely related to the penetrometer reading, had been generally considered as an important parameter for cheese quality. Effect of starter cultures type on UF processed cheese firmness was monitored using penetrometer readings (mm) and shown in Fig. 4. Principal component analysis (PCA) is reported in Fig. 5. It was found that the maximum firmness (lowest penetrometer reading) value was attributed to PC22-3 treatment which had the highest viscosity as mentioned above. On the other hand, the minimum firmness (highest penetrometer reading) was recorded by treatment PC8-1 which had the lowest viscosity. Therefore, the firmness of UF processed cheese was affected by the fermentation time, using starter cultures for one day, which indicates a decrease in the strength of the cheese network.

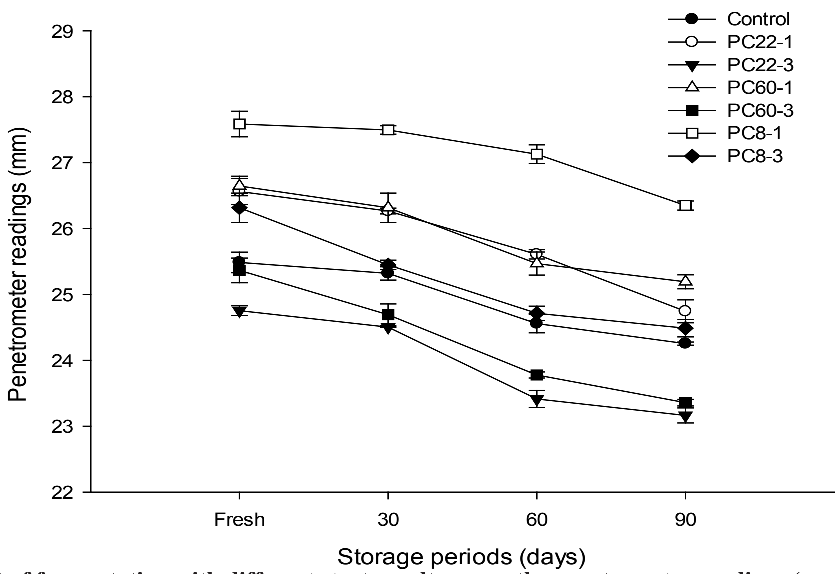

Fig. 4. Effect of fermentation with different starter cultures on the penetrometer readings (mm) of UF spreadable processed cheese during cold storage.

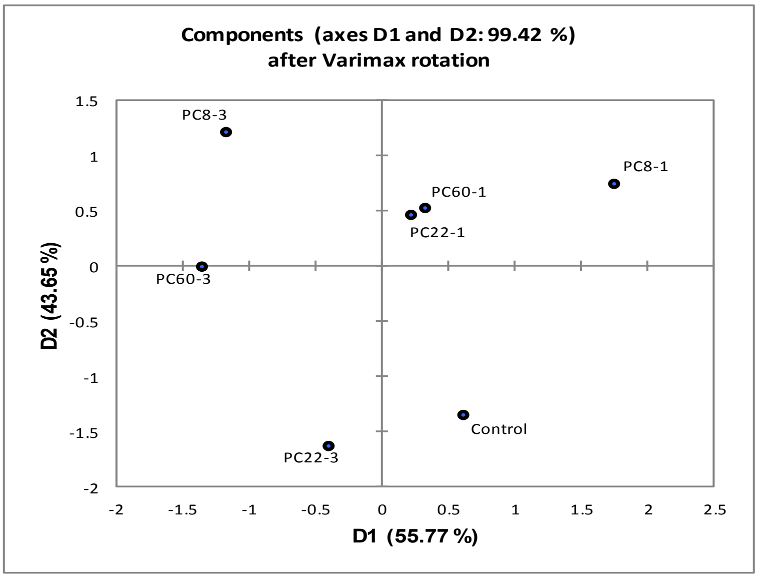

Fig 5. Principle component analysis for the penetrometer readings (mm) of UF spreadable processed cheese fermented using different starter cultures.

The PCA analysis supported and demonstrated the cheese firmness results as seen in Fig. 5, as the treatments have been divided into three categories. The first category was lying at the top right of Fig. 5 including PC8-1, PC60-1, and PC22-1 treatments incubated with starter cultures for one day, that had the lowest firmness. While the second category represented by the control treatment is in the lower right corner of Fig. 5. Finally, the third group was on the left of Fig. 5, including PC8-3, PC60-3, and PC22-3 treatments with the highest firmness, which is, most likely, due to evaporation during the incubation period. The results of the study are in concurrence with El-Garhi et al. (2018). The firmness of the treatments increased in association with the incubation time and storage period up to 90 days due to the reduction in moisture content and the acidity increase. This finding has been confirmed by several researchers who had found an inverse relationship between firmness and cheese moisture content (Amantea et al., 1986; Gupta & Reuter, 1993; Omrani Khiabanian et al., 2020). Therefore, the highest firmness was detected in treatments that fermented for three days.

Melting resistance

Melting properties influence consumer acceptance of processed cheese. Meltability is an essential functional property of cheese; particularly if it is used as a topping or an ingredient in processed foods (Hennelly et al., 2005). Table 3 presents the impact of the starter culture type and the incubation time on the meltability of the UF spreadable processed cheeses when fresh and stored for 90 days measured by modified Schreiber test. Results showed good melting properties (Schreiber test no.>4) for all starter culture types, along with the control. Meltability of fresh cheese tested by ANOVA was significantly affected ($P<0.001$) by the type of starter culture used (Table 4). Lower meltability was found with control sample than those containing starter cultures (LSD= 0.0517 at α= 0.05).

It was noticed that the meltability of the processed cheese meaningfully increased ($P<0.001$) with increasing incubation period. However, all the cheese types that were incubated with the starter cultures for 3 days showed a satisfactory meltability (Schreiber test no.>6). This was attributed to the effect of the starter cultures on the protein structure of the cheese and the acidity of the final product. Sołowiej et al. (2016) reported similar results in their study. The cheese meltability showed significant ($P<0.001$) increase along the storage period. This increase was noticeable in the cheese samples that were incubated using the starter cultures for 3 days (PC22-3, PC60-3 and PC80-3 in order). This could be due to the changes in pH and SN content of UF processed cheese (El-Garhi et al., 2018). Similar outcomes were reported by Ahmed, (2014). UF processed cheese obtained from ABT-8 starter cultures (PC80-3 and PC80-1) exhibited the highest meltability (7.78 and 7.79, respectively) compared to other starter cultures.

TABLE 3. Effect of starter cultures type and incubation time on meltability (Schreiber test number) of UF processed cheese during storage at refrigerator (6±2°C).

Treatments	Storage periods (months)			
	Fresh	1 Months	2 Months	3 Months
Control	5.28±0.41^{T}	5.31±0.03^{T}	5.45±0.14^{R}	5.68±0.23^{P}
PC22-1	5.39±0.83^{S}	5.47±0.08^{R}	5.79±0.32^{O}	6.22±0.43^{N}
PC22-3	6.18±0.93^{N}	6.44±0.26^{L}	6.83±0.39^{H}	7.11±0.28^{E}
PC60-1	5.58±1.02^{Q}	5.79±0.21^{O}	6.28±0.49^{M}	6.60±0.32^{J}
PC60-3	6.54±1.07^{K}	6.79±0.25^{H}	7.31±0.52^{D}	7.61±0.30^{B}
PC8-1	6.62±1.16^{J}	6.89±2.27^{G}	7.41±0.52^{C}	7.78±0.37^{a}
PC8-3	6.69±1.11^{I}	7.04±2.65^{F}	7.44±0.42^{C}	7.79±0.32^{a}

Note: Means ± St. Dev; different superscripts letters indicate significant difference (P<0.001). Means with different letters within a raw are significantly different from each other at =0.05 as determined by LSD range tests.

TABLE 4. Analysis of variance (ANOVA) of starter cultures type, incubation time and storage periods of UF spreadable processed cheese.

Source	DF	MS	F value	Prob	R^2
type of starter culture & fermentation (A)	6	6.568	11305.9476	0.0000	0.924
Storage (B)	3	3.611	6216.0943	0.0000	
AB	18	0.045	77.9228	0.0000	
Error	56	0.001			

Coefficient of Variation: 0.37%

Conclusions

The study of the rheological properties of UF spreadable processed cheese as affected by starter cultures types with different incubation periods helped in choosing the best treatment to give good quality cheese in structure, spreadability and meltability properties. Measuring the flow behaviour index was helpful indicating a better spreadability of the cheese made of UF milk. Additionally, viscosity measurement explained the structure of the cheese to decide on the best incubation and storage periods, which led to better-quality cheese. Moreover, it was an indication to reduce the storage period when increasing the storage temperature. Nevertheless, consistency test was a sign for fermentation with starter cultures which denoted that incubation for one day is better than 3 days for the making of processed cheese in order to avoid strong structure indicated by the high consistency values obtained. The control treatment, made without fermentation, had lower meltability values than that of treatments fermented by starter cultures, which indicated the usefulness of using the starter cultures in making the cheese. In conclusion, UF spreadable processed cheese could be manufactured from UF retentate by using thermophilic starter cultures *(Lactobacillus acidophilus, Bifidobacterium bifidus, Streptococcus salivarius* subsp. *thermophilus*) with incubation time of one day at 25 °C to improve its texture and increase meltability.

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تأثير نوع المزارع البكتيرية على الخصائص التركيبية والريولوجية وقابلية الإنصهار للجبن المطبوخ القابل للفرد المصنوع من اللبن المركز بالترشيح الفائق

نوع المزارع البكتيرية لها تأثير كبير على الخصائص الهيكلية للجبن المطبوخ القابل للفرد المصنوع من اللبن المركز بالترشيح الفائق ويمكن أن يقلل من تكلفة المنتج النهائي. يهدف هذه البحث، إلى دراسة تأثير نوع المزارع البكتيرية المستخدمة على قابلية الإنصهار والصلابة والخصائص الريولوجية للجبن المطبوخ القابل للفرد المصنوع من اللبن المركز بالترشيح الفائق (UF). تم تلقيح الجبن المطبوخ بثلاث مزارع بكتيرية هى ABT-8, CHN-22 and FRC-60. تم تحضين نصف العينات الملقحة بالمزارع البكتيرية على 25 م° لمدة 24 ساعة بينما تم تحضين النصف الأخر على 25 م° لمدة 72 ساعة. كما تم تحضير معاملة الكنترول بدون إضافة أى مزارع بكتيرية. تم فحص الخواص الريولوجية والصلابة وقابلية الإنصهار للجبن المطبوخ خلال ثلاث شهور من التخزين على 2±6°م. أظهرت النتائج وجود فروق معنوية فى مؤشرات سلوك التدفق نتيجة لإضافة مزارع البادىء المختلفة أثناء التخزين وكانت أعلى قيمة للمعاملة PC22-3 . وجد أيضا أن أعلى إنخفاض فى قيم معامل التماسك (k) كان للمعاملة PC8-1 و PC60-1طوال فترة التخزين. إنخفضت لزوجة الجبن المطبوخ نتيجة لزيادة معدل القص فى جميع المعاملات. علاوة على ذلك، أظهرت جميع العينات المختبرة أنها تتبع نموذج Herschel-Bulkley والخاص بالبسيدوبلاستيك الذى تقل فيه اللزوجة بزيادة معدل القص حيث تكون العلاقة بين قيم القص ومعدل التشوه غير خطية مع كسر هيكل الجبن بسرعة بعد القص الأولى وعلى الرغم من ذلك، إنخفض معدل الإنهيار عند رفع معدل القص. كانت المعاملات المتخمرة بالمزارع البكتيرية (PC8-1, PC22-1 and PC60-1) لمدة 24 ساعة أقل صلابة من الكنترول وباقى المعاملات الاخرى. تم الحصول على قابلية إنصهار جيدة لجميع معاملات مزارع البادىء إستجابة لوقت التخمير ومدة التخزين.