INFLUENCE OF TOTAL SOLUBLE CONTENT, STARTER CULTURE AND TIME PERIOD ON RHEOLOGICAL BEHAVIOUR OF CULTURED BUTTERMILK

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Abstract: *Casein gels are part of the dairy products, hard or soft products, as cheese and yogurt, cultured buttermilk, and their rheological behaviour can be measured by various instrumental techniques. For this study we used three types of milk (7.5%, 10%, and 12.5% respectively TSS) and starter culture (Lactococcus lactis subsp. lactis and Lactococcus lactis subsp. cremoris) in different concentrations (2 g/100 l, 3 g/100 l, 4 g/100 l). The rheological properties of the cultured buttermilk obtained from milk were investigated by a Brookfield RV Pro II+ viscometer at different shear rates, during the time period keeping (from the 1st day – the obtaining day, till the 20th day – the last day when the product is good for human consumption). The samples with the highest TSS (total soluble content) present the highest viscosity, while the samples with 7.5% TSS content present the smallest viscosity. During the rheological tests, the samples with 3 g/100 l starter culture presented the best rheological properties (the samples had the highest viscosity), while the samples with 4 g/100 l had the smallest viscosity due to the proteolysis generated by the starter culture activity. The flow index behaviour was influenced more by the TSS content rather than by the starter culture dose. It can be seen that the change in the apparent viscosity was not linear with solids concentration, where a 5% (from 7.5% to 12.5%) increase in the solids concentration led to triplicate in the apparent viscosity.*

Keywords: *viscometer, shear stress, shear rate, flow index*

1. Introduction

Rheology is the study of deformation and flow of matter. The science of rheology grew considerably due to research work done on synthetic polymers and their solutions in different solvents that in turn was necessary due to the many uses of the polymers in day-to-day and industrial applications. Nevertheless, because of the biological nature of foods, food rheology offers many unique opportunities of study. Many foods are composed mainly of biopolymers and aqueous solutions containing dissolved sugar and ions. The former ones are large molecules, often called macromolecules, such as proteins, polysaccharides, and lipids from a wide range of plant and animal sources. [1]
Rheological properties are based on flow deformation responses of foods when subjected to normal and tangential stresses [2]. In food research, the term is often used interchangeably with texture, which refers to the flow, deformation, and disintegration of a sample under force. Strictly speaking, texture relates to solid foods, and viscosity—the tendency to resist flow—relates to fluid foods. Food can exhibit both solid and liquid characteristics, and rheology can identify the properties of such foods [3].
Typically, milk is fortified with dairy ingredients to produce a milk base which is then submitted to a drastic heat treatment, which results in a high level of thermal denaturation of the whey proteins and their partial fixation on the casein micelles. As a

consequence, aggregation is promoted, giving stronger gels and decreasing the extent of acidification required to allow association. Finally, the lactic acid production during the fermentation step results in the destabilization of the micellar system and associated gelation of the proteins. As the isoelectric point of denatured proteins (pH 5.2), and casein (pH 4.6) are reached, low-energy bonds, mainly hydrophobic, are progressively established between the proteins [4]. Casein gels are responsible for many rheological properties of cheese, yogurt, buttermilk, cream and other dairy products that gel, stretch, and fracture. Rheological studies are performed as a quality control method in dairy plants and as a technique for scientists to study the structure of the product [5].

The dairy products (yogurt, cultured buttermilk, sana cultured buttermilk) presents the thixotropy phenomena. Thixotropy is the property of certain gels or fluids that are thick (viscous) under normal conditions, but flow (become thin, less viscous) over time when shaken, agitated, or otherwise stressed. Thixotropy should be defined as: the continuous decrease of viscosity with time when flow is applied to a sample that has been previously at rest and the subsequent recovery of viscosity in time when the flow is discontinued. This is consistent with the IUPAC terminology [6]. It should be noted that various general scientific dictionaries and encyclopedias still give different definitions, often more closely in line with Freundlich's original definition [7]. The essential elements of the definition used nowadays are that: it is based on viscosity; it implies a time-dependent decrease of the viscosity induced by flow; the effect is reversible when the flow is decreased or arrested [8].

The members of *Lactococcus* gen are Gram positive lactic bacteria, having a coccus form or swerved form from it, which can be oval. The cells, which can be associated in pairs or chains, have a diameter between 0,5-1,5 µm and do not present mobility. *Lactococcus lactis lactis* and *Lactococcus lactis cremoris* are mesopilic, and are characterised by their potential to produce lactic acid. They have the capacity to ferment lactose under 0,5% and have a reduce acidotolerance (are inhibited at pH<4.5) [9].

2. Materials and methods

2.1. Materials

Milk with a TSS 7,5 %, 10%, 12,5% reconstitute, *Lactococcus lactis subsp. lactis* and *subsp. cremoris* pure starter culture (Enzymes & Derivates S.A. Costisa Neamt); orbital shaker; thermostat; Brookfield viscometer Model RV- DV II Pro, with disk spindle, RV2 type.

Tab. 1.

Milk properties

	7,5%	10%	12,5%
Fat, g/100g	2.1	2.8	3.5
Protein, g/100 g	1.92	2.56	3.2
Sugar, g/100g	2.7	3.6	4.5
Ash, %	0.43	0.57	0.72
Acidity, ^{0}T	18	18	18

2.2. Sample preparation

All the samples were made using reconstituted milk. The starter culture is extracted from the freezer and thawed. The milk was heated at 90 ^{0}C for 5 min, afterwards the samples are cold to 28 ^{0}C. The starter culture was inoculated at 28 ^{0}C (2 g, 3 g and respectively 4 g starter culture /100 l milk with 7.5%, 10% and respectively 12.5% TSS). For a good activation of the starter culture, the samples are shaken for 15 min on a orbital shaker at 150 rpm. The starter culture was inoculated at 28 ^{0}C. All the samples were maintained at 28 ^{0}C for 12 h in the thermostat. The samples were kept in the refrigerator at 4 ^{0}C for 20 days.

2.3. Determination of rheological properties of cultured buttermilk

Viscosity measurements were carried out on the cultured buttermilk samples at room temperature (20^0C), with a Brookfield viscometer (Brookfield Engineering Inc, Model RV- DV II Pro+) at 0,5; 1; 2; 2,5; 4; 5, 10 and 20 rpm with RV spindle (RV2). The spindle nos (spindle nos: 2) was used in accordance with the sample nature to get all readings within the scale.

The samples in 300 ml of beaker with a 8,56 cm diameter (according to the Brookfield requests) were kept in a thermostatically controlled water bath for about 10 min before measurements in order to attain the desirable temperature of 25^0C.

First measurements were taken 2 min after the spindle was immersed in each sample, so as to allow thermal equilibrium in the sample, and to eliminate the effect of immediate time dependence.

All data were then taken after 40 s in each sample. Each measurement was duplicated on the sample.

The obtained empirical data were converted using the Mitschka relationships to shear rate and shear stress. The shear rate versus shear stress data were interpreted using the power law expression $\sigma=k\cdot\gamma^n$, where σ – shear stress (N/m^2), γ is the shear rate (s^{-1}), n is the flow behaviour index, *k* is the consistency index (Ns^n/m^2).

The values for the flow behaviour index n, were obtained from plots of log shear stress versus log rotational speed; the slope of the line (if the dependence is sufficiently close to a linear one) is simply equal to the flow index of the fluid, *n*.

3. Results and discussion

Viscosity data showed that all samples under examination were non-Newtonian fluids, since the values for the flow behaviour index, *n* were under 1, which was indicative of the thixotropy nature of culture buttermilk. The power law equation was found to be an adequate model to describe the flow behaviour of the samples in this study. The R^2 values of the equations log shear rate versus log rotational speed were found to vary from 0.950 to 0.998. The flow index behaviour (*n*) of the power law model varied between 0.208 to 0.255. Although *n* does not have a strong dependence on the concentration of starter culture, while the TSS concentration has a great impact on the index flow behaviour.

According to Mitschka, P., Steffe, J.& Daubert, C. [10, 11] the Brookfield viscometer with disk spindles represents a good method to determine non-Newtonian fluid properties.

One of the manifestations of thixotropy is the hysteresis loop. The hysteresis technique was introduced by Green and Weltmann [12]. It consists in systematically increasing and decreasing the shear rate between zero and a maximum value.

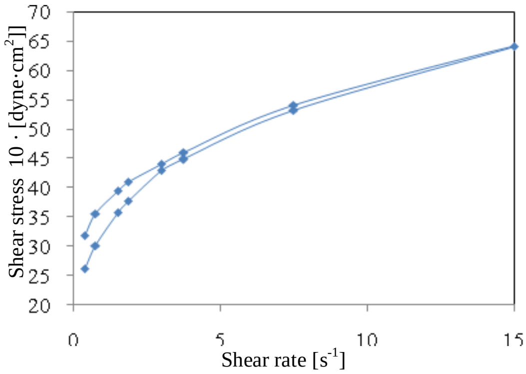

Fig.1. Hysteresis loop of culture buttermilk

The change can be a continuous ramp or a series of small steps. When the transient data are plotted as shear stress versus shear rate, a thixotropic sample will describe a hysteresis loop (fig.1) because the stress will lag behind the shear rate [8]. The gap between the two signals leads from the breakdown of the casein chain during the strain application.

The empirical data were converted to shear rate and shear stress.

The shear stress is calculated using the next equation:

$$\tau_i = k_\tau \cdot \alpha_i \cdot C \qquad [1]$$

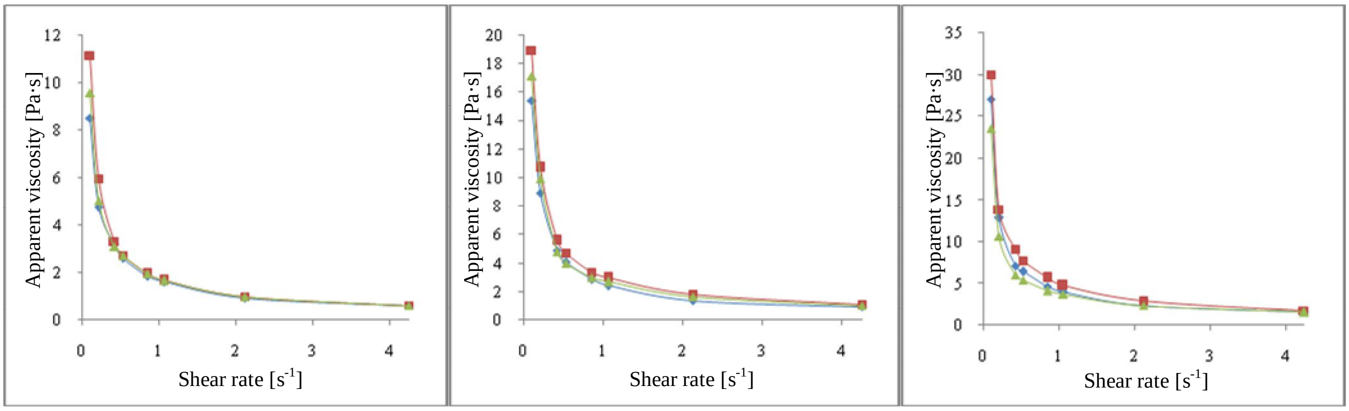

Fig. 3 Changes in the apparent viscosity with shear rate in cultured buttermilk: A. 7.5% TSS; B. 10% TSS; C. 12.5% TSS:
2 g starter culture / 100 l milk; 3g starter culture/100 l milk; 4 g starter culture / 100 l milk
***The apparent viscosity measuring were made for the 1st day**

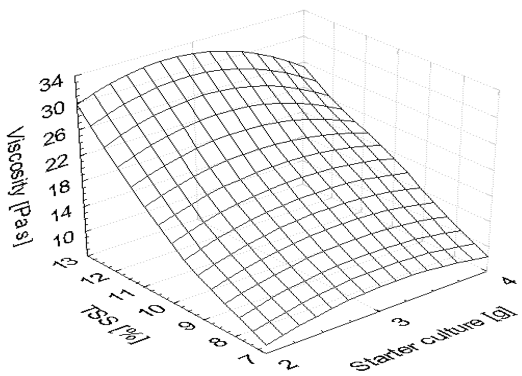

Fig. 2. Changes in the viscosity in cultured buttermilk (1st day)

Where

τ_i – shear stress [dyne/cm^2]

k_τ= 0.119, this constant is for the spindle nos 2

α_i – torque dial, %

C – 7,187 dyne/cm for RV viscometer

The shear rate is calculated using the next equation:

$$\gamma_i = k_\gamma(n) \cdot N_i \qquad [2]$$

γ_i – shear rate, s^{-1}

$k_\gamma(n)$ – constant, depends by the value of n

N_i – rotational speed, rpm

The influence of TSS on the rheology of culture buttermilk is a positive one, the viscosity increases with the increasing of TSS percent, due to the quantity of protein in the sample. The samples with 12.5% TSS had the highest viscosity, while the samples with 7.5% TSS the lowest viscosity (fig. 2), the shear stress had the same evolution like the viscosity did. The flow index behaviour varies between 0.211 and 0.255 due to the increasing of protein content of the samples.

It can be seen that the change in the apparent viscosity was not linear with solids concentration, the same observation was obtained by Hazim et al. [13], where a 5% (from 7.5% to 12.5%) increase in the solids concentration led to triplicate in the apparent viscosity (from 11.14 to 30.05 Pa·s at 0.5 rpm). This is an indication that the control of the solids concentration is an important quality factor and it may affect the final acceptance of cultured buttermilk by the consumer.

The changes of the viscosity with the starter culture dose is not a linear one, the increasing of the dose from 2 g to 3 g increased the viscosity and the shear rate (tab.2, fig.4), but from 3 g to 4 g the viscosity and the shear rate decreased. The maximum of rheological behaviour

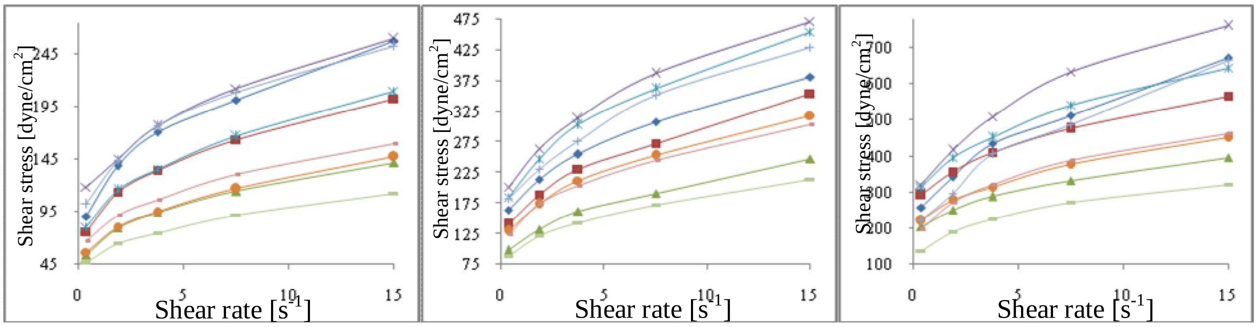

Fig. 4 Changes in the shear stress with the shear rate in the cultured buttermilk: 2 g starter culture /100 l milk 1st day; 3g starter culture/100 l milk 1st day; 4 g starter culture /100l milk 1st day; 2 g starter culture /100 l milk 10th day; 3g starter culture /100 l milk 10th day; 4 g starter culture / 100 l milk 10th day; 2 g starter culture /100 l milk 20th day ; 3g starter culture /100 l milk 20th day ; 4 g starter culture / 100 l milk 20th day

(viscosity, shear rates, flow index) is observed at 3 g starter culture as is presented by the producers on the starter culture description sheet. The decreasing of the rheological properties can be generated by the activity of the cells of *Lactococcus lactis subsp. lactis* and *subsp. Cremoris*, which is a power full proteolysis activity.

The starter culture activity increased form the 1st day to the 20th day, and the viability of the cell increased in all the samples and led to the decreasing of shear stress for all the samples due to the break of the casein chains by the activity of cells (fig.4).

Tab. 2.
Changes in the rheological properties of the cultured buttermilk prepared in three different formulations

Sample		Flow index [n]*	Apparent viscosity [Pa·s]**
7.5% TSS	2 g/100 l	0.211	8.49
	3 g/100 l	0.216	11.14
	4 g/100 l	0.208	9.57
10 % TSS	2 g/100 l	0.229	15.41
	3 g/100 l	0.234	18.89
	4 g/100 l	0.227	17.13
12.5% TSS	2 g/100 l	0.238	27.07
	3 g/100 l	0.255	30.05
	4 g/100 l	0.235	23.53

*The flow index is for the samples with 3 g/100l starter culture, in the 1st day
**The apparent viscosity was measured at 0.5 rpm

Conclusions

The rheological properties of cultered buttermilk are influeced by the TSS, starter culture and time period keeping, leading to the conclusions that the samples with 12.5% TSS and 3 g starter culture/100 l milk have the best rheology (viscosity and shear stress functions are the highest for these samples).

The Brookfield viscometer Model RV- DV II Pro+, represents a cheap method to determine rheological properties of non-Newtonian fluids, providing information about viscosity, flow index behaviour, shear rate and shear stress of fluids.

The flow index behaviour (n) varies between 0.211 and 0.255, the variation is greater with the increasing of the TSS content compared with the starter culture dose. It can be seen that the change in the apparent viscosity was not linear with solids concentration, where a 5% (from 7.5% to 12.5%) increase in the solids concentration led to triplicate in the apparent viscosity. This is an indication that the control of the solids concentration is an important quality factor and it may affect the final acceptance of cultured buttermilk by the consumer.

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