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# Rheological monitoring of structure evolution and development in stirred yoghurt

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**Abstract**

The objective of the present work was to study the rheological changes of yoghurt at different stages in the manufacturing process and to try to gain some measure of understanding of the morphological changes that occur. Rheological measurements were made on yoghurt samples for different post-sampling periods, allowing the study of the influence of time in the structure and flow properties of the material to be performed. This was accomplished using both steady and oscillatory rheometry. Up and down shear rate sweeps, constant and oscillatory shear measurements were carried out. The study of the effect of temperature allowed the observation of two distinct regions of temperature dependency, in all samples, the transition point being approximately 25°C. The structural and rheological changes that occurred during post-incubation were observed during the analysis of several yoghurt samples, indicating that there is a partial structure recovery upon cessation of flow. © 2000 Elsevier Science Ltd. All rights reserved.

## 1. Introduction

In yoghurt manufacture, the lactic acid bacteria *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* added to milk produce lactic acid from milk sugar lactose. Manufacturing methods vary considerably depending on the type of product being manufactured, raw materials used and product formulation, but there are a number of common principles that determine the nature and quality of the final product. Among these is the fortification of milk solids, the thermal treatment of the milk, the inoculation of the thermally treated milk with the bacterial culture, the incubation of the inoculated milk, the cooling of the coagulum and the packaging and chilled storage (Tamime & Robinson, 1988; Staff, 1998).

Yoghurt is usually classified in two basic types, according to its physical state in the retail container: set yoghurt and stirred yoghurt.

Set yoghurt is fermented in a retail container, which is filled after milk inoculation and is incubated in a incubation room at a suitable temperature, normally 40−43°C for approximately $2\frac{1}{2}$–4 h.

In stirred yoghurt, milk is inoculated and incubated in a fermentation tank, the yoghurt gel being broken up during the stirring, cooling and packaging stages.

Variations in the rheological properties of stirred yoghurt may be due to several factors. These can be of a physical nature such as those related with total solid content, milk composition and type of starter culture, or processing conditions-related, such as homogenisation, thermal pre-treatment of the milk and post-incubation stages (including: stirring, pumping, cooling and packaging).

The processing conditions have a great influence in the characteristics of the coagulii. Milk homogenisation is characterised by the breaking up of the milk fat globules into smaller sized ones. This improves the consistency and viscosity mainly because it prevents the rise of milk fat to the surface in the incubation tanks or in the retail container (it reduces syneresis and the tendency of the small fat globules to coalesce, because fat becomes coated with casein).

Thermal treatment of milk (95°C for 5 min) induces the main changes in the manufacture of yoghurt, because it denatures the whey proteins and induces interactions between the k-casein, β-lactoglobulin and α-lactalbumin, thus increasing the hydrophilic properties of the coagulum and the stability of the yoghurt gel (Tamime & Deeth, 1980; Tamime & Robinson, 1988).

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Once thermally treated milk has been inoculated, the starter culture begins to produce lactic acid; the resulting decrease in pH lowers the net negative charge of the casein particles and the colloidal calcium, which binds the casein micelles together, is leached out into the serum. Thus, the micelles begin to aggregate and eventually coagulate into a network of small chains as the casein is precipitated. At a certain pH, coagulation is initiated and as pH approaches the isoelectric point of casein (pH 4.6–4.7) maximum curd firmness is obtained, entrapping the fat globules and residual serum.

The protein network has interstitial spaces containing the liquid phase and it also has void spaces in which starter culture is present. After incubation, the coagulum of stirred yoghurt is broken up mechanically before cooling and packaging, thus inducing considerable changes in the rheological properties.

Benezech and Maingonnat (1992) studied the influence of cooling conditions on the flow properties of stirred yoghurt, observing that even at low wall shearing conditions during cooling the viscosity reduction is significant.

Several studies have been reported on yoghurt gels and several methods have been introduced to evaluate their rheological properties (Arshad, Paulsson & Dejmek, 1993; Benezech & Maingonnat, 1994; Bouzar, Cerning & Desmazeaud, 1997; Rönnegård & Dejmek, 1993).

The objective of the present work is to study the rheological changes of yoghurt at different points in the manufacturing process and to try to gain some measure of understanding of the morphological changes that occur. This will be accomplished using both steady and oscillatory rheometry. Therefore, the objective of this study is simply to demonstrate how Rheology, when correctly used and fully explored, helps to complement information obtained by other means.

## 2. Materials and methods

Samples at different stages during production of stirred yoghurt were obtained from a local dairy that supported this research. The points of sampling were the following:

IBT – yoghurt coagulum (sampling performed in the fermentation tank at the end of fermentation process).

IBA – cooled stirred yoghurt (sampling performed in a forced exit after the plate heat exchanger used in the cooling stage).

IBC – fruited stirred yoghurt (sampling performing after packaging stage, fruit addition done before packaging).

IBF – final stirred yoghurt (sampling after 3 h in the incubation room).

The stirred yoghurt contained 22.2% total solids, 3.12% protein and 1.9% fat (average values), these values being supplied by the producer.

All the samples, except those collected after the filling process, were collected by the same worker, using sterilised utensils, and were preserved in sterilised flasks. Samples were immediately cooled in an ice bath and then kept in the refrigerator, at a temperature of 5–7°C. Two yoghurt batches were sampled for this study.

The actual rheological measurements were made on yoghurt samples for different post-sampling periods (specified in Section 3), thus allowing the study of the influence of time in the structure and flow properties of the material. Up and down shear rate sweeps, constant and oscillatory shear measurements were carried out.

Most of the measurements were performed on a TA Instruments Weissenberg Rheogoniometer, using a parallel plate geometry 4 cm in diameter.

Sample loading obeyed a strict procedure. First, the sample was carefully removed, by means of a flat spoon, from mid-depth of an undisturbed yoghurt container/flask and placed on the bottom plate of the rheogoniometer. Then, small pieces of fruits were gently removed with tweezers (this procedure was only carried out in IBC and IBF yoghurt samples). The top plate was slowly lowered until the gap was between 2400 and 2500 μm, as measured by the equipment, in order not to squash the sample. The gap value varied, as it was referred before, since the slice of sample removed did not have a constant height. Therefore, in order to avoid the damage of sample by squashing, small differences in the gap value had to exist. Finally, the excess yoghurt was removed from the edges of the plate.

Up and down shearing experiments were conducted in a continuous ramp mode, the shear rate sweeps ranging from 0.001 to 100 $s^{-1}$, the ascent and descent times of 5 min, at 10°C. In experiments designed to study structure break-up under shear, the samples were sheared at a constant shear rate of 5 $s^{-1}$, during 15 min, also at 10°C.

Development of structure during manufacture of stirred yoghurt was studied by means of oscillatory rheological measurements. Initially, an amplitude sweep, at a constant frequency of 1 Hz, and displacement ranging from $1\times10^{-4}$ to 0.1 rad, was carried out, so that the viscoelastic region of the yoghurt samples could be determined. Once this was done, log frequency sweeps were conducted at a constant displacement of 0.01 rad, with oscillation frequencies ranging from 0.001 to 10 Hz. In view of the limitations of the rheogoniometer to accurately control temperature at temperatures below 20°C, for long periods (more than 5 min) these experiments were carried out at this temperature and each experiment was repeated three times.

The study of the influence of lagged time between sampling and the analysis was carried out using both steady and oscillatory shear measurements. Samples were analysed 2, 7 and 14 days after sampling. The data presented below are average results, with an average error of 4.7%.

The temperature effect in the rheological properties of yoghurt was studied by means of apparent viscosity measurements at different spindle speed rotation, at temperatures of 5°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C and 45°C, the experiments being performed using a Viscometers ELV-8 model with a TCU-3 module, using coaxial cylinders with spindle TL7. In the experiments carried out at temperatures higher than 20°C, the TCU-3 module was switched on to keep the temperature constant. Initially, the sample temperature was previously stabilised in a thermostatic bath at the temperature selected for the experiment. Simultaneously, the temperature of the TCU-3 module was stabilised during a period of approximately 30 min (this procedure was only carried out at temperatures higher than 20°C). In agreement with the equipment manual, 9.5 ml of sample was used in each experiment. After the sample loading (with a syringe) spindle speed rotation sweeps were conducted ranging from 0.3 to 60 rpm, at constant temperature. Each experiment was repeated three times.

This study was only carried out in IBA, IBC and IBF yoghurt samples, since to study IBT the yoghurt coagulum would have to be destroyed.

Also, the study was done in order to verify if the temperature difference between the steady (10°C) and oscillatory (20°C) rheometry experiments was acceptable in terms of material structure. The reason for this is the (already mentioned) fact that the Weissenberg Rheogoniometer does not control accurately the temperature below 20°C, for long time experiments (such as the ones involved in oscillatory experiments).

## 3. Results and discussion

In the study of the temperature effect in the rheological properties of stirred yoghurt it was possible to observe, as it was expected, that the apparent viscosity of the yoghurt samples decreased as the temperature increased, for all samples, this effect being more pronounced for temperatures higher than 25°C. This study allowed the application of the well-known shear rate–temperature superposition method (see, for example, Nielsen, 1977), whereby a correspondence between data obtained under certain experimental conditions, i.e., certain shear rate and temperature ranges, can be used to predict the flow behaviour of the material at other equivalent ones, i.e., at the same shear stress.

The displacement factor is mathematically defined as $a_T = \dot{\gamma}(\text{reference})/\dot{\gamma}(T)$, where $\dot{\gamma}$(reference) is the shear rate at the reference temperature at a defined shear stress and $\dot{\gamma}(T)$ is the shear rate at a determined temperature $T$, at the same shear stress. Assuming that the temperature dependency of the apparent viscosity can be characterised by the Arrhenius-type equation ($\eta = K\mathrm{e}^{E/RT}$), the displacement factor can also be defined as $a_T = \eta(T)/\eta(\text{reference})$, where $E$ is the activation energy. $E$ is an indication of material sensitivity to temperature variations.

Based on the displacement factors ($a_T$) determined for a reference temperature of 20°C, it was possible to evaluate the temperature dependency of the displacement factor through the Arrhenius equation, for all yoghurt samples, shown in Fig. 1. In this figure it is possible to observe the existence of two different regions, that express a flow behaviour showing different temperature dependencies.

The existence of two regions with different temperature dependencies is evident from the changes in the energy activation values of the samples. From the slope values ($E/R$, $R = 8.31451$ J K$^{-1}$ mol$^{-1}$) shown in Fig. 1 the activation energy values were determined, as shown in Table 1. For temperatures below 25°C, all samples showed activation energy values rather lower than those for temperatures above 25°C. The reasons for this behaviour are probably related with the re-start of the bacteria activity. In addition, the IBA sample shows a greater activation energy value than IBC and IBF, for the same temperature interval, in the lower temperature range. This difference may be due to the fact that the IBA protein structure is less disrupted than IBC and IBF, since this sample was collected in a prior stage of production.

On the basis of this weak dependency of viscosity on temperature, below 25°C, it is assumed that it is valid to perform steady and oscillatory rheometry experiments at 10°C and 20°C, respectively, since bacteria activity has not re-started yet.

In the flow curves of yoghurt samples shown in Fig. 2, it is possible to observe the thixotropic character of stirred yoghurt and the extent of structural breakdown

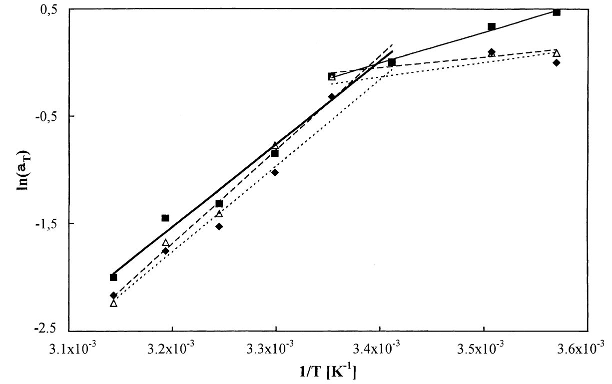

Fig. 1. Temperature dependency of displacement factor ($a_T$) for IBA, IBC and IBF yoghurt samples. Symbols are: (■) IBA; (△) IBC; (◆) IBF.

Table 1
Energy activation values, at constant shear stress, for IBA, IBC and IBF yoghurt samples

| Sample | Temperature interval (°C) | Slope (*K*) | Activation energy (J mol$^{-1}$) |
|---|---|---|---|
| IBA | 7–25 | 2907.4 | 24173.6 |
| | 25–45 | 7719.4 | 64183.0 |
| IBC | 6–25 | 1018.1 | 8465.0 |
| | 25–45 | 8783.3 | 73028.8 |
| IBF | 6–25 | 1393.4 | 11585.4 |
| | 25–45 | 8055.9 | 66980.9 |

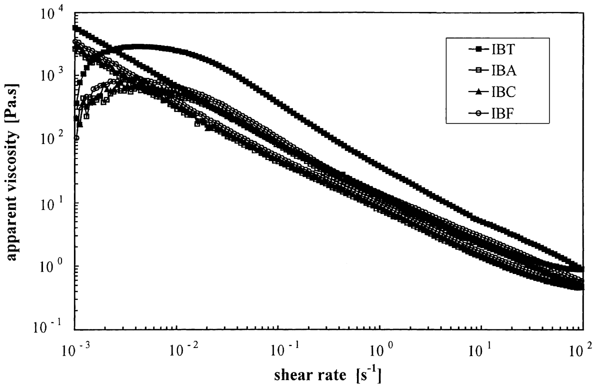

Fig. 2. Representative upward and downward flow curves of yoghurt during different stages of stirred yoghurt manufacture.

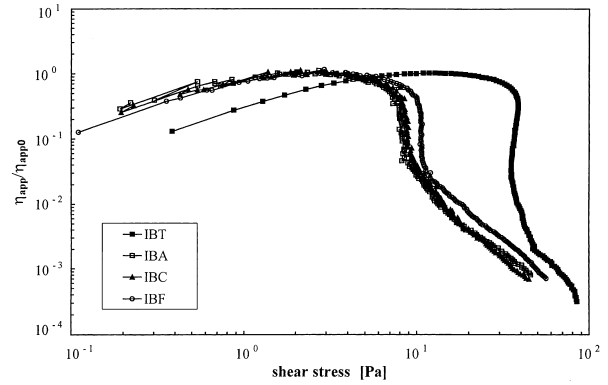

Fig. 3. Representative plot of reduced apparent viscosity ($\eta_{APP}/\eta_{APP0}$) versus shear stress, (upward cycle) of yoghurt during different stages of stirred yoghurt manufacture.

during shearing. The hysteresis loop of yoghurt coagulum (IBT) indicates that this sample was subjected to a major structural degradation during the upward cycle. This was expected since in this sample the protein network was intact. At low shear rates the viscosity increased with increasing shear because this region corresponds to a transient stress growth at start-up of steady shear. In the other samples the structure breakdown was not so significant since they were collected along the post-incubation stages (stirring, pumping, cooling and packaging) where the yoghurt gel was already broken.

The differences in structural degradation among stirred yoghurt samples can also be observed in Fig. 3 in which average plots of reduced ($\eta/\eta_0$) apparent viscosity versus shear stress during the upward cycle are shown. IBA, IBC and IBF samples have similar ($\eta/\eta_0$) behaviours. In the first two cases, the viscosity is approximately constant (excluding transient effects at the start-up of shear) up to a shear stress of approximately 8 Pa after which there is a sudden, dramatic decrease in ($\eta/\eta_0$). In the IBF yoghurt sample this sudden decrease of ($\eta/\eta_0$) occured at a shear stress of 10 Pa. This decrease is similar to that observed in systems where wall-slip is present, this point being further discussed later. The sample collected in the fermentation tank at the end of fermentation process (IBT) exhibited two points of sudden decay of ($\eta/\eta_0$), one at a shear stress of approximately 37.5 Pa and a second one at 83 Pa. These points may correspond to critical values of shear stress at which significant structure breakdowns occurs, as will be explained below. All the experiments were performed 14 days after sampling (the importance of this time lag will also be further discussed below).

The sudden decreases of apparent viscosity were similar to that observed in systems where wall-slip is present, i.e., wall depletion effects, due to the displacement of the disperse phase away from solid boundaries, leaving a lower viscosity, liquid-depleted layer (Barnes, 1995). In fact, this wall-slip effect is most likely due to the displacement of the whey away from the protein networks as the disruption of this structure occurs.

These sudden decreases of apparent viscosity are consonant with a model proposed by van Marieke (1998). In this, it is proposed that protein particles build up aggregates which in turn build up ‘super-aggregates’. The super-aggregates can be disrupted at shear rates lower than 10 s$^{-1}$, and the primary gel particles are disrupted at a shear rate range from 10 to 447 s$^{-1}$, at the used experimental conditions. Shear rates greater than 447 s$^{-1}$ result in the disruption of the primary aggregates into protein particles. This is in accordance with a model for shear-induced structural degradation of yoghurt made using starter cultures that produce exopolysaccharides (Eps) proposed by Hess, Roberts and Ziegler (1997). In this, it was shown that, as undisturbed yoghurt was sheared, there was a progressive disruption of the portion of the casein micelle network that was not associated with Eps. As shear forces were increased and exceeded a critical value the disruption of the portion of the casein network that was associated with Eps occurred.

Therefore, the two inflection points observed in IBT yoghurt sample might be explained with basis on these models; the first drop in ($\eta/\eta_0$) corresponds to the disruption of super-aggregates, where the portion of the casein micelle network that was not associated with Eps was broken. In the second point, the disruption of primary-aggregates occurred, breaking the portion of the casein network that was associated with Eps. The shear stresses at which this inflection occurs are in the shear rate range proposed by van Marieke (1998) (shear stress is the dominating factor in structure breakdown, not shear rate).

Since each day of production corresponded to a set of experiments, the reproducibility of the process was studied with base on IBA (which is the critical point of the sampling process). The results are shown in Fig. 4 and it is apparent that the process is reproducible within the experimental error.

The structure breakdown in time, for constant shear, is shown in Fig. 5, for all samples, where it is possible to assess that the general behaviour is in agreement with what could be expected in the basis of Figs. 2 and 3. Again, IBA and IBC exhibited similar behaviour, whereas significant differences for IBT are observed. IBT was the sample that took more time to reach equilibrium, since the protein network had to be disrupted first. Comparing IBC and IBF yoghurt samples, one can observe that IBF exhibited, initially, a reduced ($\eta/\eta_{eq}$) apparent viscosity higher than IBC sample, therefore one can deduce that some structure recovery occurred during the second incubation stage, to which it was submitted during production. However, after 10 min of shearing all yoghurt samples presented similar reduced apparent viscosities. These results are comparable with those obtained by Benezech and Maingonnat (1993), when viscosity decay with time was studied, at constant shear rates during 600 s, for different commercial yoghurts.

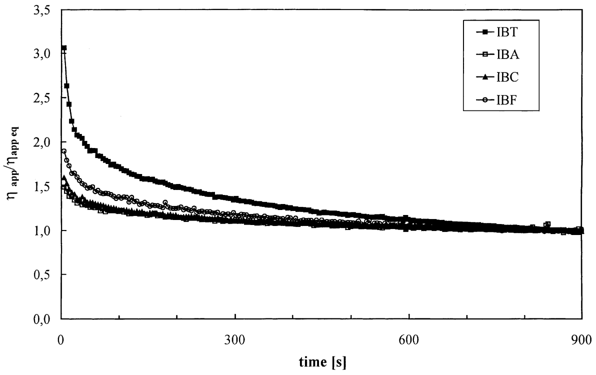

Fig. 5. Representative plots of structure degradation in time.

Structural analysis was performed by means of oscillatory shear measurements, which was done in order to assure that structure was not being destroyed as testing was being carried out. Average log frequency sweeps for all samples are shown in Fig. 6 and the results are in line with those from steady shear, the IBT sample showing significantly higher storage modulus and dynamic viscosity than the remainder. Again, in IBF it was possible to observe some degree of structural rebuilding. As far as IBA and IBC are concerned, no significant differences between the structures were observed even though IBC contained fruit concentrate.

In order to check for both reproducibility in time and possible insight into the sort of structure one is dealing with, the influence of time lagged between the sampling and the analysis was studied for all yoghurt samples. The differences of structure degradation during the ‘upward cycle’, with different storage time, for IBA, can be observed in Fig. 7, where the apparent viscosity

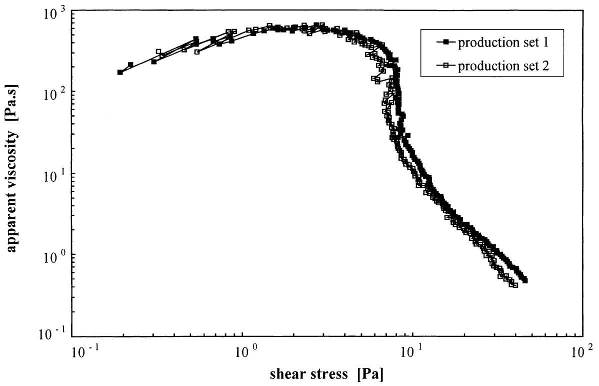

Fig. 4. Reproducibility study of IBA, for two different production sets.

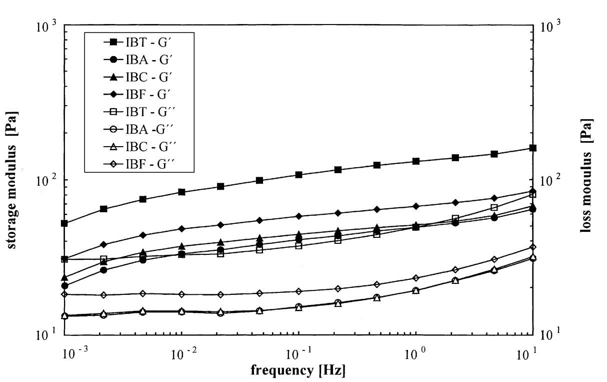

Fig. 6. Storage and loss moduli for all samples.

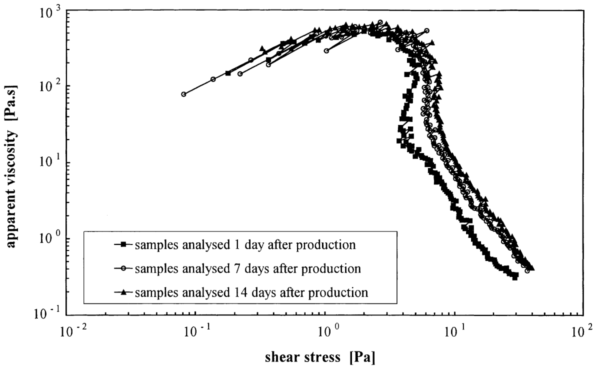

Fig. 7. Representative plot of apparent viscosity versus shear rate (upward cycle) of IBA in function of storage time.

versus shear stress plots are represented. Like in Fig. 3, a point of sudden decrease of apparent viscosity is observed. The stress at which this occurs, however, increases with time until it reaches an equilibrium value of approximately 8 Pa, as mentioned earlier (which occurs after 14 days). This effect can be related to an increase in the consistency of the yoghurt that happens due to syneresis, protein hydration and post-sampling Eps production (Rašik & Kurman, 1978).

Fig. 8 corresponds to the structural analysis of IBA by means of oscillatory shear measurements 2, 7 and 14 days after sampling. The constant values of $G'$ and $G''$ at low frequencies are characteristics in an elastic solid behaviour (Macosko, 1994). The rubber-like behaviour shown by all samples, i.e., the constant values of $G'$ and $G''$ at low frequencies and the transition of $G''$ to another constant, higher value at high frequencies, is a strong indication that some sort of weak elastic structure is present, most likely a protein network. This is so because these are the weakest interactions and, therefore, are likely to be the first to re-form. In the samples with higher storage time, i.e., 7 and 14 days, it is possible to see that this structure has been severely degraded (this can be seen by the lower values of $G'$) probably due to the occurence of some enzymatic phenomena such as after-acidification and proteolysis (Rašik & Kurman, 1978; Tamime & Deeth, 1980; Rajagopal & Sandine, 1990).

After-acidification is probably present because the enzymatic activity of lactic cultures, though reduced, is not completely stopped. Further decreases in pH induce ionic changes in the protein network, thus originating a disruption in the protein–protein interactions (Rašik & Kurman, 1978; Harwalkar & Kaláb, 1986). This disruption will promote syneresis which, in turn, originates some demineralisation, contributing to further degradation of the weak structure. The occurrence of syneresis, for long storage times, may explain the fact that a decrease in the value of $G'$ is observed at very low frequencies, i.e., very long testing times. Additionally, proteolysis will occur, further contributing to this structural degradation.

Another interesting feature of Fig. 8 is the change in the transition between *plateaus* of $G''$ (which relates to the relaxation time of the structure) to lower frequencies, i.e., higher times, from 2 to 7 days of sample storage. The yoghurt sample with the shortest storage time (2 days) shows the transition occurring at a frequency of approximately 1 Hz, whereas for 7 and 14 days this occurs at 0.1 Hz. This is so because, although structure degradation occurs, the consistency of the yoghurt increases in time. It is interesting to notice that, although the consistency is increasing, the combined effect of proteolysis and after-acidification still dominates at long storage times, causing the observed drops in $G'$ and $G''$ between 7 and 14 days storage time.

The Cox–Merz diagram presented in Fig. 9, for a reference temperature of 20°C, confirms what has been said (cf. Cox & Merz, 1958). In fact, one can observe the structural difference due to the storage time in the form of a decreasing complex viscosity, whereas the increase in consistency is shown in the slight increase in shear viscosity (refer also to Fig. 7 in this case). With an increase of the storage time, one is comparing increasingly ‘like with like’, and therefore it is to be expected that the Cox–Merz rule be approximately valid for long storage times only, as is the case.

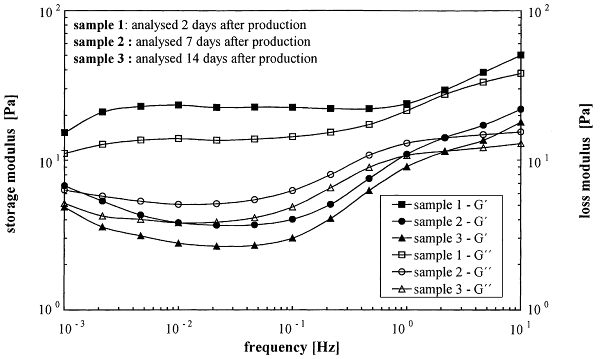

Fig. 8. Storage and loss moduli of IBA in function of storage time.

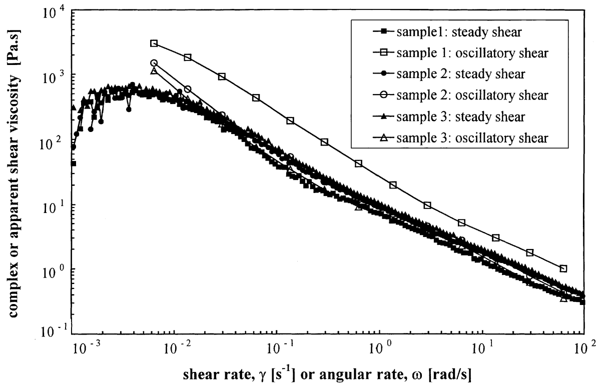

Fig. 9. Cox–Merz diagram of IBA.

The influence of storage time on the yoghurt samples was also studied for IBT, IBC and IBF and the results are shown in Figs. 10–12, respectively. In IBT, which corresponds to undisturbed yoghurt coagulum, there are no significant structural changes as storage time increases, only small differences having been observed due to the increase in consistency. This strongly contrasts with the behaviour observed for IBA (see Fig. 8).

In IBC, a significant increase in storage and loss moduli as storage time increases is observed. This happens due to the addition of fruit concentrate which results in increased consistency (due to the increase in sugar concentration and the swelling of pectins present in these concentrates). This behaviour is not observed in the IBF sample though one can verify an increase of the dynamic parameters with time, but the differences are not so significant, since this yoghurt sample was submitted to a thermal stabilisation after the addition of fruit concentrate and the packaging stage.

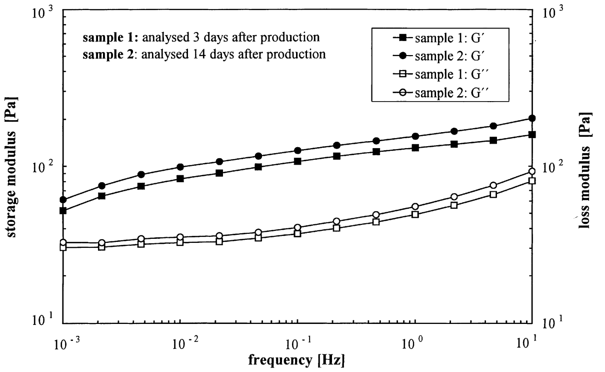

Fig. 10. Storage and loss moduli of IBT in function of storage time.

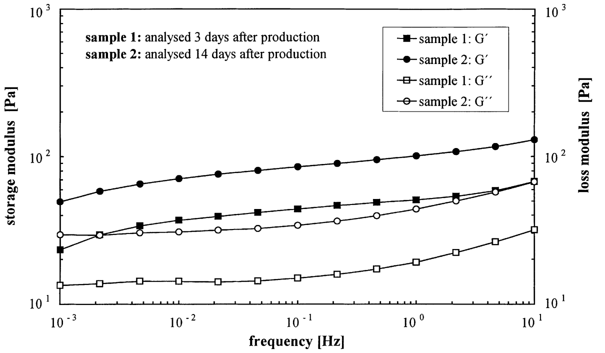

Fig. 11. Storage and loss moduli of IBC in function of storage time.

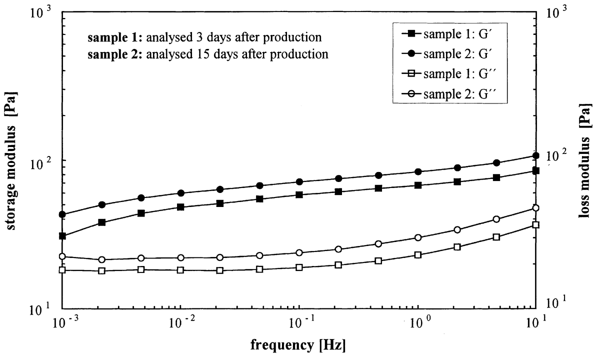

Fig. 12. Storage and loss moduli of IBF in function of storage time.

Ramaswamy and Basak (1992) studied the effect of the addition of raspberry concentrate on the rheology of stirred yoghurt concluding that the addition of concentrate resulted in increased yoghurt viscosity and shear degradability. These conclusions are consistent with the higher structure recovery observed with long storage times for IBC when compared with IBA, although the shear viscosities are very similar.

## 4. Conclusions

The aim of this study was the rheological monitoring of structure evolution and development during the production stages and storage of stirred yoghurt, which was performed by means of steady and oscillatory shear experiments.

Yoghurt collected in the fermentation tank (IBT) before the stirring stage was the one that presented the major structural degradation during shearing and minor structural changes with increased storage time, as it was expected since this sample corresponds to the undisturbed yoghurt coagulum.

The structural and rheological changes that occurred during post-incubation stages were observed during the analysis of IBA, IBC and IBF yoghurt samples, confirming that strong structural degradation occurs as shear forces are increased.

The influence of storage time was more significant in the IBA yoghurt sample in which case a weak proteic structure quickly formed. As storage time increased this structure severely degraded (due to the occurrence of after-acidification and proteolysis), which led to lower values of the storage and loss moduli although the viscosity of the material increased (due to syneresis, protein hydration and Eps production).

The effect of addition of fruit concentrate on the structure and rheological properties was studied in IBC. The results show a slight increase in apparent shear viscosity and a strong increase in the dynamic moduli, which is probably due to the increase in sugar concentration and the swelling of pectins present in these concentrates.

Temperature seems to have different effects on the flow behaviour of the materials in different temperature ranges. It is very likely that this is related to the onset of bacterial activity but, nonetheless, this subject needs further work since it is possible that more complex structural changes may be involved.

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