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Research Article

Stability of Oligosaccharides Derived from Lactose and Lactulose regarding Rheological and Thermal Properties

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Prebiotic carbohydrates derived from lactulose (OsLu) are appealing ingredients that provide beneficial effects following their fermentation by the gut microbiota; however, more investigation is needed to evaluate their technological properties and applicability as a functional food ingredient in the market. In this paper, a comparative study on the rheological and thermal behavior of OsLu and a commercial mixture of galactooligosaccharides (GOS) was carried out. In both cases, there was a strong influence of temperature and shear rate on viscosity. Viscosity of OsLu was higher than that of commercial GOS and variations and rheopexy cycles were also higher for OsLu. The exponential increase of viscosity associated with the structural changes took place later in OsLu than in commercial GOS, suggesting more stability to the shear over time in the former. More stability of OsLu was also observed in the study of the effect of temperature on viscosity. In addition, the thermal study indicated different behavior of both prebiotics under the assayed conditions, showing that OsLu have lower values of glass transition temperature (Tg) than commercial GOS and that commercial GOS are more sensible against humidity. The results here obtained provide important information on the treatment and storage conditions of these prebiotic ingredients during the elaboration of functional foods.

1. Introduction

The increasing interest toward intestinal function has promoted obtaining a wide range of oligosaccharides with enough scientific evidence of prebiotic activity [1–3]; moreover, prebiotic oligosaccharides are considered functional food components due to their outstanding technological properties that can improve the sensory features in a number of food applications. In this sense, the food industry is recently paying attention to the benefits of incorporating different prebiotic oligosaccharides in the elaboration of foodstuffs to modify texture and replace fat or as low-calorie sweetener [4, 5]. In addition, they have several applications in other fields like the pharmaceutical and cosmetic areas [6].

Among the existing oligosaccharide mixtures in the market, galactooligosaccharides (GOS) are, together with fructooligosaccharides (FOS), one of the most used, mainly in infant formula [6]. The commercially available preparations of GOS are formed by mixtures with variable composition and concentration depending on the source and charge of enzyme, initial substrate amount, temperature and time, the different formed structures determining not only their physiological effects but also their physicochemical properties, and, consequently, their rheology [1]. As it is known, the knowledge of the rheological behavior of oligosaccharide solutions may be of paramount importance in food process design, evaluation, and modeling. In addition, the rheological characteristics are even indicators of the product quality and play an important role in processes such as evaporation, drying, and pasteurization [7]. To the best of our knowledge, hardly any studies have been carried out on the rheological characteristic of GOS. According to Van Leusen et al. [8],

Table 1: Carbohydrate composition (% of total carbohydrates) of OsLu and Vivinal GOS.

	Glc	Fru	Gal	Di	Lac	Lu	Tri	Tetra	Penta	Hexa
OsLu	-	0.6 (±0.2)	14.1 (±1.0)	21.1 (±1.1)	-	26.1 (±1.2)	25.6 (±0.7)	9.7 (±0.7)	2.6 (±0.6)	0.2 (±0.1)
Vivinal GOS	20.7 (±2.1)	-	1.4 (±0.1)	20.5 (±0.6)	18.0 (±0.2)	-	21.0 (±0.7)	13.1 (±0.8)	4.8 (±0.6)	0.7 (±0.4)

Note. These values are per 100 g of carbohydrates. Standard deviation values are given in brackets.

at temperatures between 4°C and 20°C, the viscosity of GOS syrup with 75% of dry matter extract was correlated to a Newtonian behavior, that is, independent of the shear rate applied, whereas, at 50°C, a shear thinning effect was observed. The flow behavior was only studied up to shear rates of 100 s^{-1}.

Furthermore, the stability during storage of this type of preparations can facilitate their usefulness in food formulations at the industry. The stabilizing effect of GOS, which occurs at temperatures below the glass transition temperature (Tg), has been related to its capacity to originate matrices with scarce molecular interactions, although the mobility of small molecules of gases, water, and organic compounds can lead to destabilization of amorphous structures. The thermal stability of two commercial GOS (i.e., Biotempo and Cup Oligo H-70®) in comparison to lactulose has been assessed by differential scanning calorimetry (DSC). The study of Tg indicated that the vitrification capacity of different prebiotics follows the order GOS Biotempo > GOS Cup Oligo H-70 > lactulose [9].

Oligosaccharides derived from lactulose (OsLu) are a new generation of prebiotic oligosaccharides with enhanced prebiotic properties, as it has been shown in *in vitro* studies [10–12]. OsLu present different structural features as compared to GOS, highlighting the presence of a molecule of fructose instead of glucose at the reducing unit, although additional compositional differences may also rely on the degree of polymerization, anomeric configuration, isomer composition, and/or types of glycosidic linkage. These structural dissimilarities between OsLu and GOS have been associated with the fact that OsLu could reach the most distant portions of gut where most of the important gastrointestinal pathologies can take place [6, 13]. However, as it is known, the development of novel bioactive oligosaccharides will be of commercial interest only if they are formulated into real foods and placed in the market. In this sense, López-Sanz et al. [14] demonstrated that OsLu can be stable during the thermal processing and storage conditions in foods with a pH in the range 3.4–6.8. However, no studies on the rheological and thermal properties of the mixture of OsLu have been done so far.

Based on these considerations, the main objective of this work was to analyze the rheological and thermal behavior of a mixture of OsLu elaborated in the laboratory under enzymatic synthesis in comparison to a commercial preparation of GOS. To do that, the flow and viscosity (η) curves, as well as the effect of shear rate ($\dot{\gamma}$), temperature and time on viscosity (η), and the determination of Tg were addressed.

2. Materials and Methods

2.1. Samples. OsLu were obtained at pilot scale by Innaves SA (Vigo, Spain) following the method described by Anadon et al. [15] with some changes, as indicated by López-Sanz et al. [14]. OsLu were synthesised using a commercial lactulose preparation (Duphalac, Abbott Biologicals BV, Olst, Netherlands) and β-galactosidase from *Aspergillus oryzae* (16 U/mL; Sigma, St. Louis, MO). The mixture of oligosaccharides (20% [w/v]) was treated with fresh *Saccharomyces cerevisiae* (1.5% [w/v]; Levital, Paniberica de Levadura® SA, Valladolid, Spain) to remove monosaccharides. Vivinal GOS syrup was kindly provided by Friesland Campina Domo (Hanzeplein, The Netherlands). Both samples were dried at 40°C in a rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland) to reach a dry matter of 80% (±1).

2.2. Characterisation of Oligosaccharide Mixtures. Prior to rheological study, a physicochemical and compositional characterisation of oligosaccharide mixtures was carried out as it is shown in López-Sanz et al. [14]. The composition in carbohydrates (fructose, glucose, galactose, lactose, lactulose, disaccharides different from lactose and lactulose, tri-, tetra-, penta- and hexaoligosaccharides) of OsLu and Vivinal GOS syrups expressed in g per 100 g of total carbohydrates is shown in Table 1. The predominant glycosidic linkages involved in OsLu and Vivinal GOS oligosaccharides were $\beta(1\rightarrow6)$ and $\beta(1\rightarrow4)$, respectively.

2.3. Rheological Properties of OsLu and Vivinal GOS. The rheological properties of an ingredient are given by the answer offered to the shear deformation and the viscosity (η) can be affected by the shear rate ($\dot{\gamma}$), shear time, and temperature [16]. Thus, the effect of these variables on the rheological behavior of OsLu and Vivinal GOS was studied.

The rheological properties of samples were determined using a rotational rheometer Haake MARS (Haake, Thermo, USA) in controlled shear rate mode. The measurement system used was of plate-plate (PP35), namely, two parallel plane plates with a smooth surface, a diameter of 35 mm, and a gap of 1 mm between plates. To avoid variation from the centre to the rim of the plate, the program makes shear rate corrections. Due to the fact that not shear-induced agglomerates and wall-depletion phenomena were observed, the utilization of serrated surface plates was not necessary. The temperature was controlled through circulation of water below the measurement base plate from a thermostatic bath.

Due to the thermal inertia of materials, cycles were designed with the times necessary to ensure the actual temperature change imposed by the controller. Samples were cooled or heated to the desired temperature before being incorporated into the system to avoid temperature gradients due to the sample. The software used for evaluation of the data was Rheo Win 4 Job Manager.

2.3.1. Effect of Shear Rate. The variation of shear stress (σ) and viscosity (η) was evaluated in controlled shear rate ($\dot{\gamma}$) mode. The conditions were chosen taking into account the previous studies on rheology of GOS [8]. The measurement conditions were as follows: phase 1, increasing the shear rate between 0 and 500 s^{-1} in 180 s; phase 2, constant shear rate (500 s^{-1}) during 60 s; and phase 3, decreasing the shear rate between 500 and 0 s^{-1} in 180 s. The flow curves and the viscosity curves were determined at 25, 35, 50, and 70°C. Furthermore, other measurement was carried out with a maximum shear rate of 10 s^{-1} and at 4°C.

2.3.2. Effect of Shearing Time. The variation of η versus time during shearing was evaluated at 25°C. The measurement conditions were as follows: phase 1, increasing the shear rate between 0 and 10 s^{-1} in 30 s; phase 2, constant shear rate (10 s^{-1}) during 600 s; and phase 3, decreasing the shear rate between 10 and 0 s^{-1} in 30 s. This low value of shear rate was selected to prevent excessive shearing on the samples that could destroy their possible structure.

2.3.3. Effect of Temperature. In addition to the viscosity curves measured at several temperatures, the variation of viscosity as a function of temperature was also measured by designing a specific measuring cycle at constant shear rate. The measurement conditions were as follows: phase 1, increasing the shear rate between 0 and 10 s^{-1} at 25°C in 30 s; phase 2, constant shear rate (10 s^{-1}) between 25 and 80°C during 3600 s; and phase 3, decreasing the shear rate between 10 and 0 s^{-1} at 80°C in 30 s. Samples were covered with a thin plastic ring to prevent water evaporation.

2.4. Thermal Behavior of OsLu and Vivinal GOS. The thermal stability of the prebiotic mixtures was performed in the freeze-dried samples (2 mg, approximately) [9] by differential scanning calorimetry (DSC) and thermogravimetry (TGA) flushing N_2 at 50 and 90 mL/min, respectively. In the former, the determinations were carried out in a TA Instrument Discovery DSC using heating rates of 1, 5, 10, and 20°C/min from −80 to 200°C and three subsequent cycles of heating and cooling. TGA analyses were done in a thermobalance TA Instruments TGA Q500 equipped with an EGA oven, using 10°C/min as heating rate.

3. Results and Discussion

3.1. Effect of Shear Rate on Viscosity. Figure 1 illustrates the flow curves (σ versus $\dot{\gamma}$) of OsLu and Vivinal GOS, measured at different temperatures. The corresponding viscosity curves (η versus $\dot{\gamma}$) are shown in Figure 2. The viscosity curves are plotted in log-log scale in order to allow the easy evaluation of the possible shear thickening behavior of some samples.

The curves shown correspond only to the increasing shear rate ramp of the measurement since it was observed that after shearing the samples developed a structure, so that viscosity increased in an uncontrolled way and the descending ramp was, in most cases, very irregular shaped and had not a continuous trend associated with any rheological model. In all cases the measurements revealed a complex behavior with large time-dependent hysteresis cycles and an apparent rheopexy. This complex behavior consisted of the following steps: (i) a shear thinning response for increasing shear rates; (ii) a marked increase of viscosity on shearing above a certain shear rate that depends on the temperature; (iii) an uncontrolled increase of viscosity during shearing at the maximum shear rate, that is, 500 s^{-1}; and (iv) a shear thinning behavior in the down-curve in which the viscosities are higher than in the up-curve. This could be associated with the creation of a severe structure in the sample as a consequence of shearing, and the irregularities of the down-curve (not shown) demonstrated the increased heterogeneity in the sample. To avoid the possible errors associated with these irregular curves obtained with sheared and hence modified samples, the further analysis was performed considered only the increasing shear rate stage of the measurements. The rheological behaviors of both samples (OsLu and Vivinal GOS) were strongly influenced by temperature and shear rate, although in the case of Vivinal GOS the viscosity was lower and, consequently, the hysteresis cycles.

At 4°C, in the shear rate range applied (0–10 s^{-1}), the flow and viscosity curves of OsLu and Vivinal GOS were very close to Newtonian; namely, both curves had a linear behavior (Figures 1 and 2); nevertheless, a small hysteresis cycle was detected in the case of Vivinal GOS. These results suggested hardly any effect of shear on structural change of both syrups at 4°C (Figures 1 and 2). However, in the flow and viscosity curves of OsLu and Vivinal GOS at temperatures of 25–50°C within a shear rate range of 0–500 s^{-1}, important variations were observed, and the behavior of both ingredients became shear thinning indicating structural changes due to the shear that increased with the rise of temperature. When temperature increased above 50°C the rheological behavior changed and the up-ramp of the flow curves became shear thickening, effect that was more evident as temperature raised. This change from shear thinning to shear thickening behavior observed for increasing temperature is clearly appreciated in the log-log viscosity.

In general, viscosity of OsLu was higher than that of Vivinal GOS (Figures 1 and 2); therefore, variations and rheopexy cycles were also higher for OsLu, probably ascribed to the differences in the carbohydrate composition, since OsLu present higher concentration in compounds of DP $\geq$ 2, as shown in Table 1. In addition, in both cases, OsLu and Vivinal GOS samples, viscosity decreased with increasing of shear rate. However, the presence of irregularities associated with structure formation seemed to be larger for Vivinal GOS, thus suggesting the lower stability of this sample.

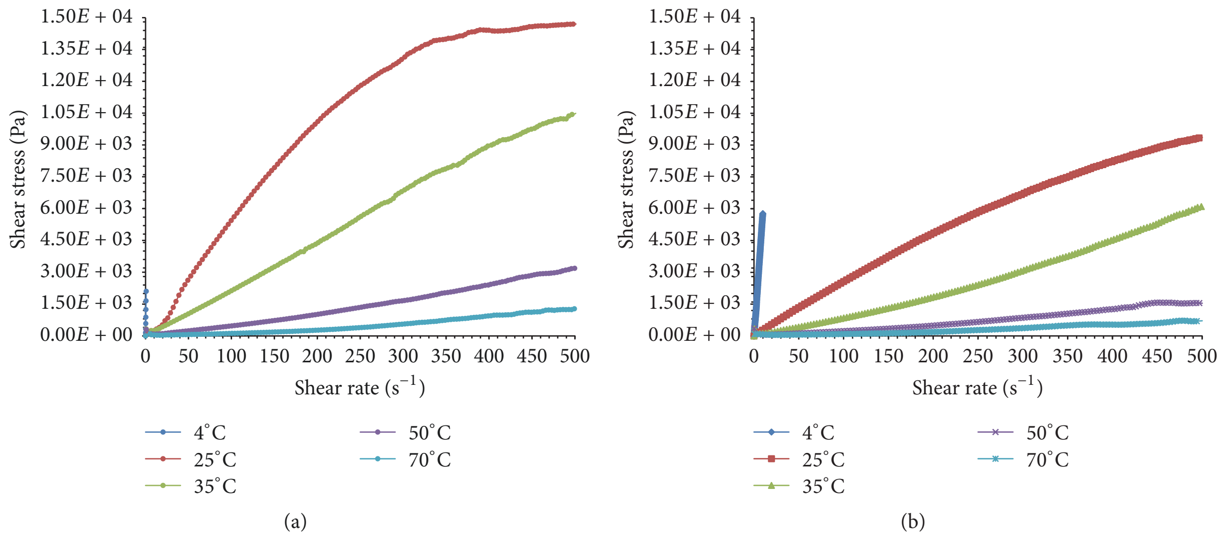

FIGURE 1: Flow curves of OsLu (a) and Vivinal GOS (b) at different temperatures: 4, 25, 35, 50, and 70°C.

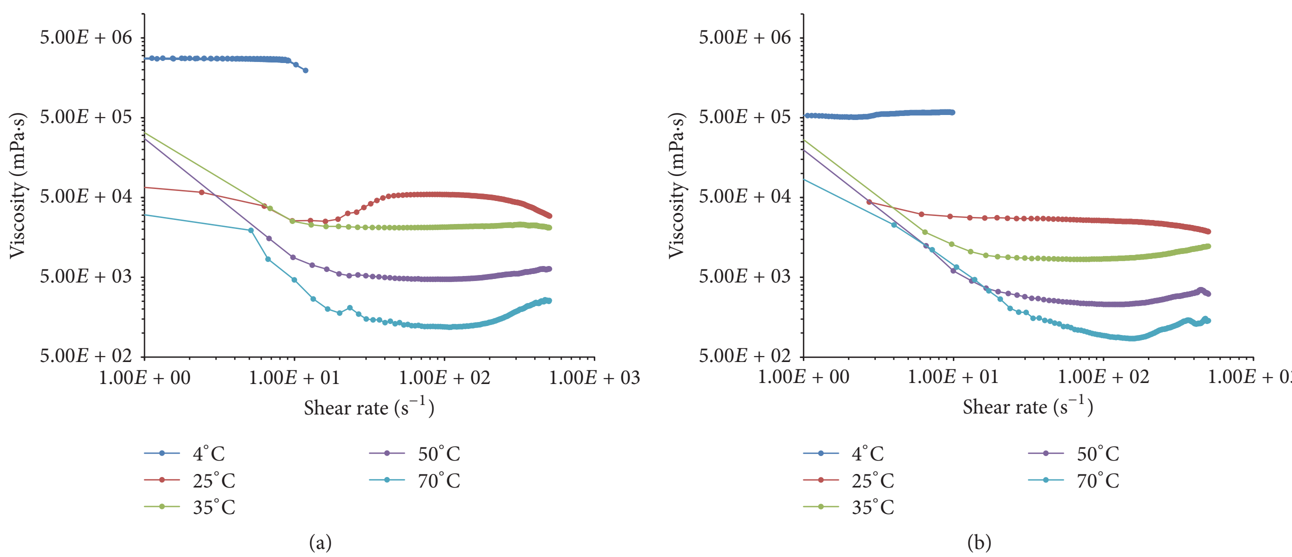

FIGURE 2: Viscosity curves of OsLu (a) and Vivinal GOS (b) at different temperatures: 4, 25, 35, 50, and 70°C.

As aforementioned, to the best of our knowledge, hardly any studies have been carried out on the rheological characteristic of prebiotic oligosaccharides. Van Leusen et al. [8], in a study on viscosity of GOS syrup (75% DM) at temperatures between 4 and 20°C, observed that the viscosity was independent of shear rate. These differences could be due, among other factors, to the different time periods evaluated, since in the study of Van Leusen et al. [8] shear rate was only investigated up to rates of 100 s^{-1}, whereas in our study the change from shear thinning to shear thickening occurs at higher shear rates. Toneli et al. [7] studied the rheological properties of concentrated inulin and they found a highly pseudoplastic behavior for all the evaluated soluble solids concentrations with high resistance to flow at low shear rates followed by a breakdown of the structure when shear rate increased.

3.2. Effect of Shearing Time on Viscosity. The variation of viscosity with time at constant shear rate (10 s^{-1}) and temperature (25°C) of OsLu and Vivinal GOS was also studied, as it is shown in Figure 3. In both ingredients, viscosity remains nearly constant with time until an exponential and sharp increase of viscosity is registered at a particular time indicating the formation of a rigid structure due to the fact that shearing promotes the formation of a network structure associated with the modification of the sample, which confirms the rheopectic cycle observed in the flow curve measurements. In the case of OsLu, the exponential increase of viscosity associated with the structural changes took place later than in Vivinal GOS ingredient (~800 s versus ~620 s, resp.), suggesting again that OsLu maintains stable for longer time than Vivinal GOS under low shearing.

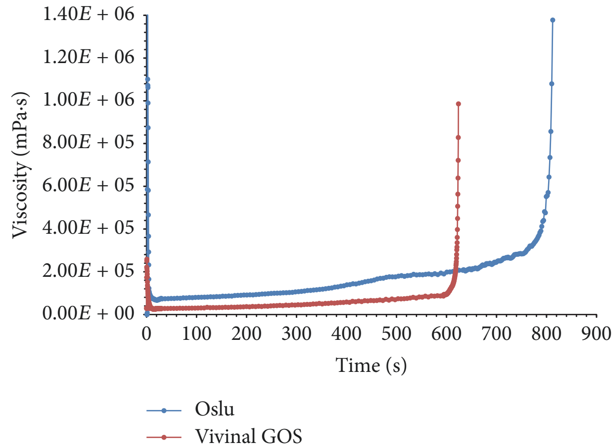

Figure 3: Variation of viscosity with time at constant shear rate (10 s^{-1}) and temperature (25°C).

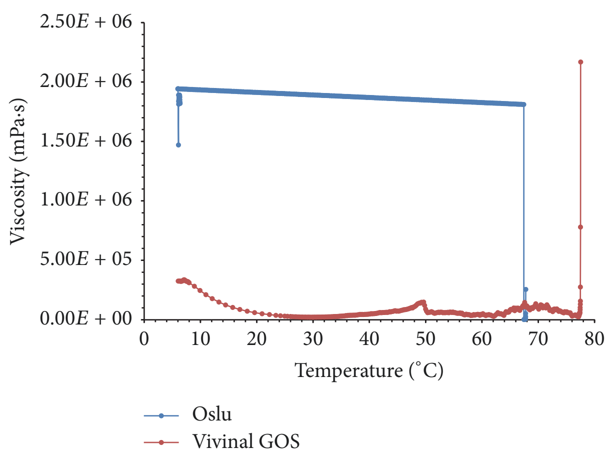

Figure 4: Variation of viscosity with temperature at constant shear rate (10 s^{-1}) during 1 h.

Since limited rheological studies of such syrups have been reported, it is difficult to compare these results with data from the literature. For this reason, due to the high content in carbohydrates (close to 80%), a comparison with other syrup such as honey was tried. Yanniotis et al. [17] carried out a study on effect of time on viscosity of several honeys. Viscosity was measured at constant shear rate (40 s^{-1}) as function of time (0 to 40 min) at 45°C, and they observed that viscosity was not time-dependent. Probably the higher concentration of oligosaccharides in OsLu and Vivinal GOS syrups than honey could affect, among other factors, this type of measurements. Once more the previous studies did not reach the shear rate conditions necessary to produce the structural changes observed in our work

3.3. Effect of Continuous Heating on Viscosity. Figure 4 depicts the variation of viscosity of OsLu and Vivinal GOS syrups with increasing temperature at constant shear rate (10 s^{-1}) during 1 h. As shown in Sections 3.1 and 3.2, viscosity of OsLu sample was considerably greater than Vivinal GOS and decreased very slightly and uniformly with temperature. However, in Vivinal GOS, this decrease was noticeable up to approximately 30°C, temperature at which viscosity started to increase and, at 50°C, a heterogeneous behavior was detected. These changes in viscosity with temperature could also indicate structural changes in the components, probably due to the hydrolysis of high molecular weight molecules [7]. Therefore, similar to the above indicated data, OsLu seemed to be more stable against temperature than Vivinal GOS, from a rheological perspective.

Oroian [18] carried out a study on effect of temperature on the viscosity of several honeys, the temperature range studied being 20–50°C. In samples with an initial value of 100–100000 mPa·s, a decrease in viscosity with the increasing of temperature was also observed, in good agreement with the behavior observed for the OsLu syrup.

3.4. Thermal Properties of OsLu and Vivinal GOS. Initially, a DSC analysis of both OsLu and Vivinal GOS was carried out with a heating rate of 10°C/min (Figure 5). In the DSC curve of OsLu (Figure 5(a)), Tg close to 22°C and a decrease in the baseline, probably due to the presence of volatile compounds, were observed together with a temperature of decomposition near 140°C. Similar trend was detected in the case of Vivinal GOS (Figure 5(b)), with a possible Tg close to 36°C and a decomposition temperature of 135°C, although, in this case, an endothermic peak (fusion or volatile loss) at 90°C, approximately, was also observed.

In order to more accurately identify the different signals and temperatures of both prebiotic mixtures and try to obtain some information about the storage behavior of the freeze-dried compounds, additional DSC assays were done with other heating rates (1, 5, and 20°C/min), as shown in Figures 6 and 7 (only heating curves were included since cooling ones did not provide additional information). Regarding OsLu (Figure 6) and Vivinal GOS (Figure 7), similar thermal behaviors were found with a decrease corresponding to Tg and also an endothermic peak at the slowest heating rates (1 and 5°C), probably ascribed to loss of water. At 1 and 5°C/min of heating rates, the endothermic peaks of curves of Vivinal GOS presented minima values of 82.5 and 85.5°C,

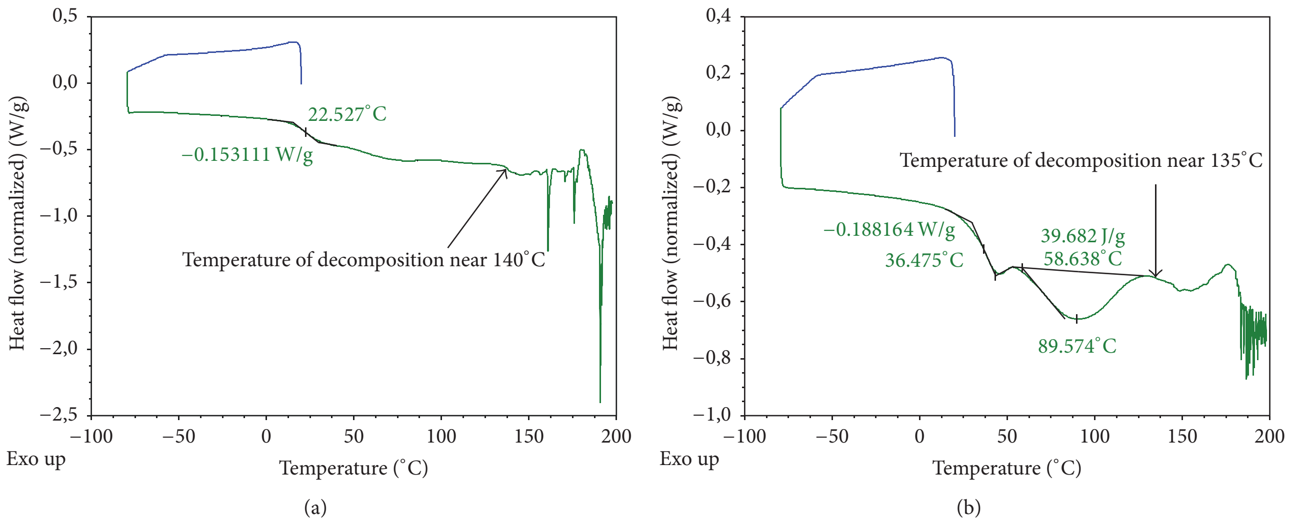

Figure 5: DSC curves corresponding to OsLu (a) and Vivinal GOS (b), using a heating rate of 10°C/min and one cycle of heating and cooling.

Table 2: *Tg* values obtained after the analysis of OsLu and Vivinal GOS by DSC using three heating rates and three subsequent cycles of heating and cooling.

	Heating rate (°C/min)	*Tg* (°C) Cycle 1	Cycle 2	Cycle 3
OsLu	1	17.1	57.1	61.3
	5	12.3	16.3	19.7
	20	−2.3	8.8	11.0
Vivinal GOS	1	26.7	82.0	85.3
	5	13.9	57.0	61.2
	20	5.0	24.1	28.6

respectively, whereas in OsLu they were much lower (67.2 and 72.0°C), indicating that both prebiotic ingredients have different distribution of water.

Comparing the three heating/cooling cycles in each graph, *Tg* was shifted to higher temperature values, this displacement being higher at the lowest heating rate (1°C/min). This result is due to the fact that the presence of water exerts a plastifying effect with the consequent decrease in *Tg*. Thus, the lower the heating rate is, the best the drying effect was found in the sample. As observed in Table 2, *Tg* values were lower in OsLu than in Vivinal GOS, indicating that the former should be stored at lower temperatures than the latter. This could be due to the higher composition in oligosaccharides of higher DP (from 4 to 6) in Vivinal GOS than in OsLu, as indicated in Table 1 [9].

In addition, TGA analysis was also carried out in both prebiotic mixtures. Table 3 shows a quite different behavior of OsLu and Vivinal GOS. The percentage of the first loss of weight was the half in OsLu compared to that in Vivinal GOS (1.4 versus 3.1%). Moreover, the temperature at which this loss was finished was lower in the former (90 versus 116°C). It is reasonable to attribute this loss to adsorption of water. In the second interval of temperature, the loss of weight can be due to absorbed water with the following proportions: 3.3% for OsLu (90–157°C) and 2.1% in Vivinal GOS (116–170°C). This is in agreement with the higher value of viscosity in the former than in the latter, as aforementioned (Section 3.1.), since in a more viscous syrup it is more difficult to carry out the freeze-drying process (samples for thermal study were freeze-dried). At higher temperatures the decomposition processes were similar in OsLu and Vivinal GOS, despite being with quantitative differences in the percentages of weight loss. Taken into account these assays, Vivinal GOS seems to be more prone to humidity than OsLu, particularly at the lowest interval of temperature analysed.

4. Conclusions

At the sight of the results here shown it is possible to conclude that the rheological behavior of both OsLu and Vivinal GOS syrups was strongly influenced by the shear rate, shearing time, and temperature. Viscosity was higher in OsLu than in a commercial GOS mixture and increased with time and decreased with shear rate and temperature. However, all rheological parameters evaluated indicated that OsLu syrup is more stable than Vivinal GOS against temperature changes. In relation to the thermal study carried out in freeze-dried samples, the obtained results seem to indicate that although freeze-dried OsLu should be stored at lower temperatures than Vivinal GOS, the latter is more susceptible to the humidity. To our knowledge, this is the first investigation on the rheological and thermal behavior of OsLu. Although further studies on food formulation should be conducted, these data should be considered during the application of prebiotic carbohydrates in food processing.

Conflicts of Interest

The authors have no conflicts of interest.

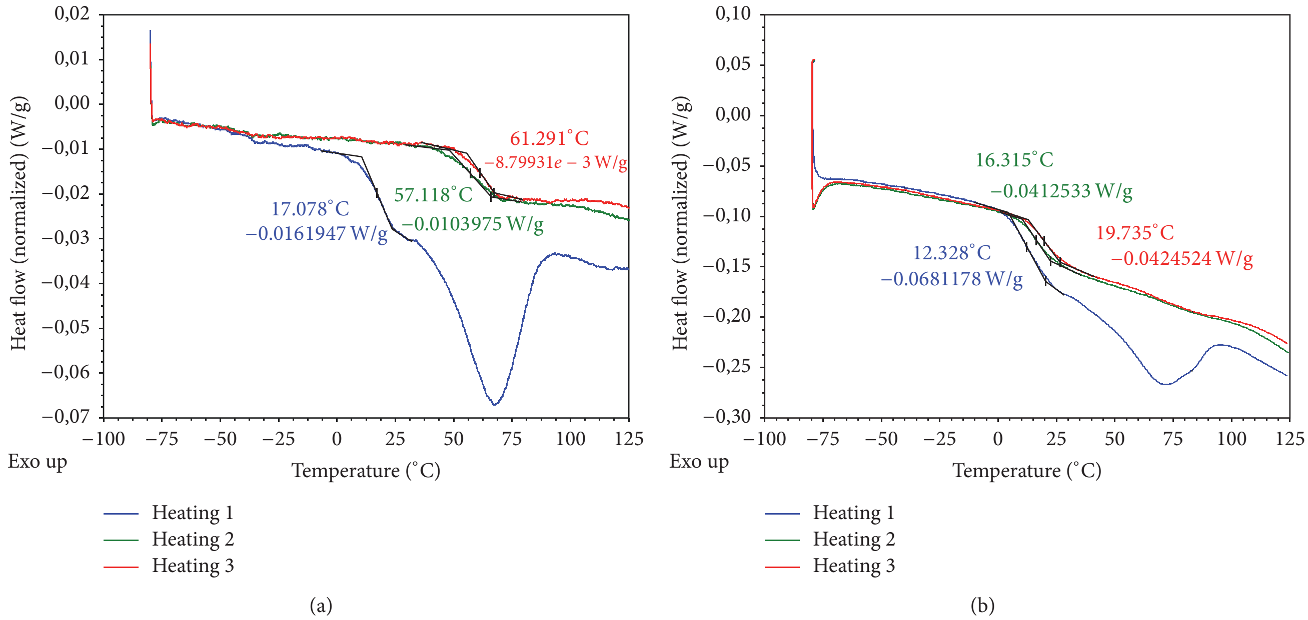

Figure 6: DSC curves of OsLu using heating rates of (a) 1, (b) 5, and (c) 20°C/min and three subsequent cycles of heating and cooling.

Table 3: Percentages of weight loss and weight accumulated obtained from the TGA curves of OsLu and Vivinal GOS.

	Range of temperature (°C)	Weight loss (%)	Weight accumulated (%)
OsLu	25–90	1.4	98.4
	90–157	3.3	95.2
	157–252	25.6	69.6
	252–900	52.2	17.4
Vivinal GOS	25–116	3.1	96.9
	116–170	2.1	94.8
	170–252	16.1	76.9
	252–900	60.4	18.2

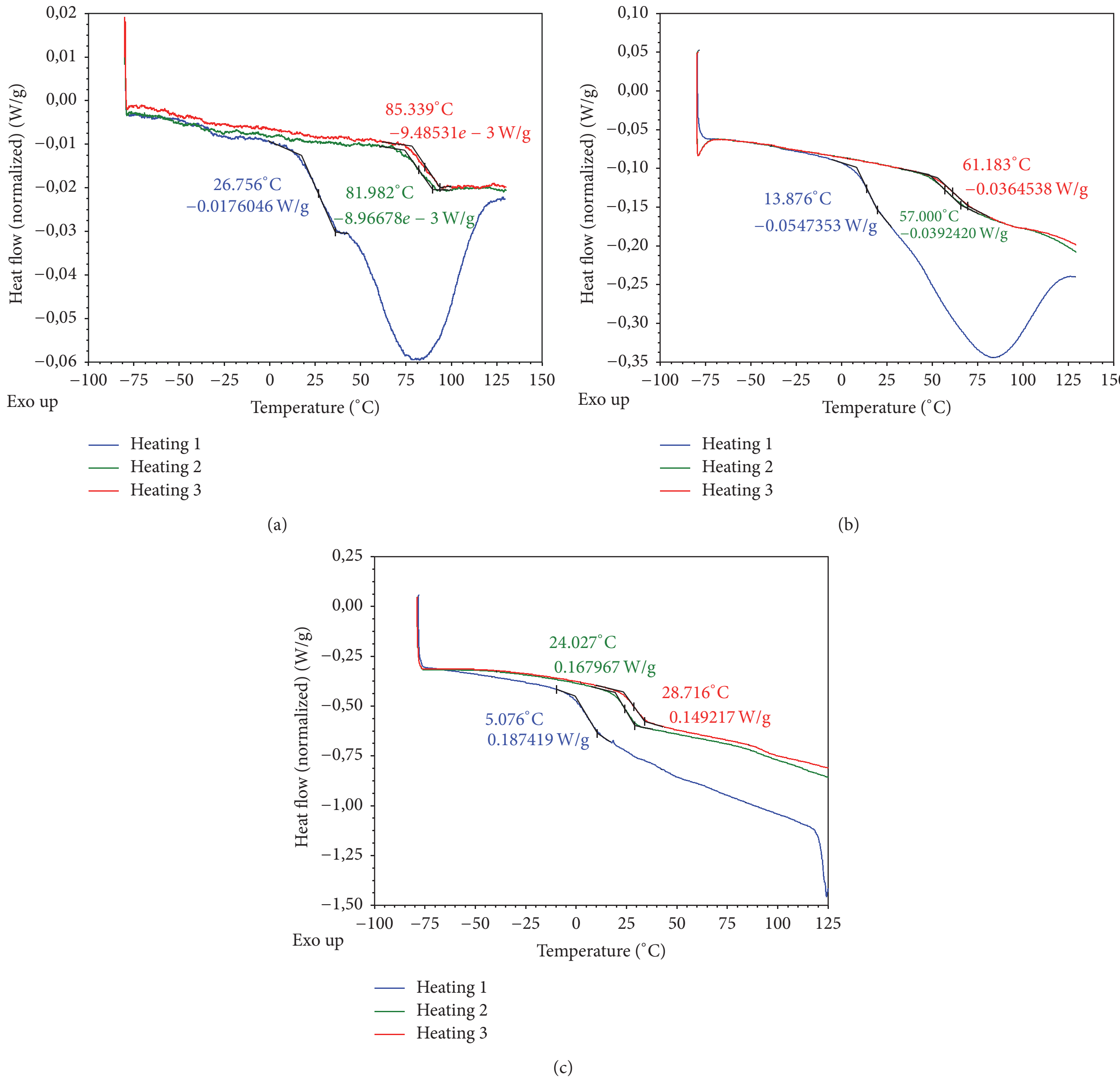

FIGURE 7: DSC curves of Vivinal GOS using heating rates of (a) 1, (b) 5, and (c) 20°C/min and three subsequent cycles of heating and cooling.

Acknowledgments

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