Dry-Heat Treatment vs. Heat-Moisture Treatment assisted by microwave radiation: Techno-functional and rheological modifications of rice flour

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Abstract

The capacity of microwave (MW)-assisted dry-heat and heat-moisture treatments to modify techno-functional properties and gel viscoelasticity of rice flour was studied. Flour samples at 30%, 20%, 15%, 13%, 8%, and 3% of moisture content (MC) were microwaved for 480 s at 18W/g. The 3% MC sample was unmodified by treatment. However, the 8% MC sample was one of the most affected, along with the 20% and 30% samples, indicating that a small amount of water can plasticize rice flour enough to rearrange its biopolymers molecules (mainly starch) responsible for the modifications. The lowest peak and breakdown viscosities and the highest pasting temperature were found in the 30% MC sample, which varied -58%, -89%, and +8ºC respectively, from native flour. DSC revealed partial pre-gelatinization in the 20% and 30% MC samples. Swelling power increased in all samples after treatment, while water solubility only increased in the 8% and 30% MC samples. Treatments promoted gel stability and enhanced viscoelastic moduli, particularly in the 8% and 20% MC samples. Flour MC in MW-assisted treatment allowed the modulation of techno-functional properties of rice flour and rheological and thermal characteristics of their gels.

***Keywords**: Rice flour; Microwave radiation; Techno-functional properties; Pasting properties; Gel Rheological properties.*

1. Introduction

Research efforts in the manufacture of gluten-free (GF) products have rapidly increased over the last decade. Various approaches are being used to improve the quality of these products, which generally have poor sensory properties. Physical methods such as heat moisture treatments and annealing appear to be good alternatives to improve gluten free dough and bread characteristics (Villanueva, Harasym, Muñoz, & Ronda, 2019). They are considered to be safer and more environmentally friendly compared to chemical modification (Oyeyinka, Umaru, Olatunde, & Joseph, 2019).

Heat-moisture treatment (HMT) is by far the most studied method of physical modification of starch. Typically, HMT utilizes high moisture contents (20-35 %) and temperatures (>90 °C) for long treatment periods (1-24 h). However, dry-heat treatment (DHT) has recently received a great deal of attention because of its simplicity and safety. DHT is a physical modification that can affect the starch granules to a certain extent and change the structure of the crystalline and amorphous regions of starch and, as a consequence, its physicochemical properties. The usual DHT conditions include the use of low moisture levels (<10 % w/w) and high temperatures (>110 °C) for 1-4 h (K. Liu, Hao, Chen, & Gao, 2019). It is assumed that in both HMT and DHT the structure of the starch granules is not destroyed (Braşoveanu & Nemţanu, 2014).

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world. In addition to whole rice, global rice flour production has increased significantly due to its hypoallergenic proteins and lack of gluten, which make it essential in the formulation of GF products (Villanueva, Harasym, Muñoz, & Ronda, 2018). Many works have studied the physical modification of rice flour by HMT (moisture content of 20-35%) (Puncha-arnon & Uttapap, 2013). Recently, the effects of DHT on the structure and physicochemical properties of rice starch have also been investigated (Kyung, Young, & Gyu, 2018). The effect of both treatments, HMT and DHT, depends on several variables such as type of starch, moisture content (MC), temperature, and time of heating (BeMiller, 2018; Braşoveanu & Nemţanu, 2014). Almost universally, HMT decreases starch solubility, swelling power, amylose leaching, and peak viscosity, and increases pasting temperature (Srikaeo, Ruiz, Srikaeo, & Segovia de la Revilla, 2018). In the case of DHT, it seems to increase gel strength and pasting viscosity above 130 ºC, at short treatment periods, whereas both were reduced with increasing heating time. Furthermore, the amylose content of rice starches influenced their pasting, physicochemical, and rheological properties after DHT (Kyung et al., 2018).

Traditional heating systems used in both HMT and DHT are often expensive and difficult to implement on a large scale; so alternative methods, such as microwave (MW) heating, have been investigated (Su, Chotineeranat, Laoka, & Chatakanonda, 2018). It provides fast, uniform heating, and it is economical and environmentally friendly, as it minimizes energy consumption (Sun, Xu, & Xiong, 2014). It has demonstrated effects on the structure and physicochemical properties of cereal, tuber, and legume starches (Braşoveanu & Nemţanu, 2014). Several factors such as MW time and power, MC, and botanical source of flour may significantly influence the physicochemical properties of the treated flour (Oyeyinka et al., 2019). Most of the literature refers to MW heating of starches under HMT conditions, while very few studies applied DHT conditions, with water content <15 %, even though it was shown that low moisture starch samples underwent transformation differently from high moisture samples (Lewicka, Siemion, & Kurcok, 2015). Most of the work found in literature used commercial MW equipment, where the flour was irradiated in non-hermetic containers (Braşoveanu & Nemţanu, 2014). Although the evolution of the flour MC during treatment is generally not reported, it has been seen that it is quickly lost over time, reaching very low values, close to the mono-layer water content of the flour, after some few minutes of treatment (Villanueva, Harasym, et al., 2018). Therefore, MW treatments are often mixed HM-DH treatments that do not facilitate establishing the exact role of sample MC in the changes experienced, since it varies over time. In this study the rice flour MC was kept constant during MW radiation using a hermetic container and MCs ranging from 3 % (freeze-dried sample) to 30 %. The effect of MC during MW treatment on the physicochemical and pasting properties of rice flour have been evaluated for the first time to establish the relative importance of the plasticizing effect of water and the temperature reached by the flour during treatment in modifying these properties. The MC values tested ranged from 3 % and 8 % (very low/low values that could be classified within DHT processes) to 20 % and 30 % (high values, classified within the HMT processes) passing through 13 % and 15 % (natural MC of flour and intermedium value located in the border between DHT and HMT) The impact of treatment on the thermal and viscoelastic properties of gels made with microwaved-flours was also established.

2. Materials and methods

2.1 Samples

Indica rice (long grain) flour was kindly provided by Herba Ricemills S.L.U. (Valencia, Spain). The MC was 13 %, ash < 0.9 %, protein > 6.5 %, fat < 1 %, and gluten < 10 ppm. The flour granulometry was: > 250 µm (1 %), 210-250 µm (10-20 %), 150-210 µm (35-45 %), 100-150 µm (20-35 %), and < 100 µm (10-20 %) (data provided by manufacturer).

2.2 Preparation of heat-treated rice flour

Rice flour MC was measured with the AACC 44-19.01 Official Method (AACC, 1999). Six batches at different MCs of 3 %, 8 %, 13 %, 15 %, 20 %, and 30 % ± 0.5 % were prepared. The MC of 3% was obtained by rice flour lyophilization in FreeZone 1, Labconco lyophilizer (Kansas City, USA). The MC of 8 % was obtained by drying the native flour in an incubation chamber (Memmert ICP260, Schwabach, Germany) at 40 ºC until the desired MC was achieved. The natural rice flour MC was 13 %. The water needed to reach the levels of 15 %, 20 %, and 30 % was added as was described by Villanueva, Harasym, et al. (2018)

2.3 Microwave treatment

A 900 W Sharp MW oven (R342INW) (Sakai, Japan) was used to perform the MW treatments. 50 g of flour at desired MC levels were exposed to MW radiation (100 % power) for 480 s in cycles of 10 s radiation and 60 s of rest in a hermetic Teflon® container (volume of 1000 mL). It was constantly stirred during treatment as was described previously (Villanueva, Harasym, et al., 2018). The temperature reached in each treatment was measured by Testoterm® temperature strips from TESTO (Barcelona, Spain), which were placed in the container in permanent contact with the flour sample following the procedure described by Villanueva, Harasym, et al. (2018). After cooling to room temperature, the samples were sieved to <250 µm for further analysis. The MC of the samples was checked after treatment and it was verified that the MC did not change more than 0.5 % from its initial value. Samples at 20 % and 30 % MC were dried at 35 ºC until getting back to 13 % to avoid spoilage. Untreated rice flour was used as control. Each treatment was carried out in triplicate.

2.4 Apparent amylose content

Apparent amylose content (AAC) of native and microwaved flours was determined by the lectin concanavalin A method (Gibson, Solah, & McCleary, 1997) using a K-AMYL assay kit (Megazyme International Ltd., Ireland). The absorbance was read at 510 nm. Three replicates were made for each sample.

2.5 Hydration properties

Water absorption capacity (WAC), water absorption index (WAI), water solubility index (WSI), and swelling power (SP) of rice flour samples were measured. Two grams of each sample were mixed with 20 mL of distilled water in 50 mL centrifuge tubes. WAC (g water retained/g flour dry matter [dm]) was determined following Abebe, Collar, and Ronda (2015). WAI, WSI, and SP of the flours were calculated as described by Abebe et al. (2015), with slight modifications. The dispersions were cooked for 15 min at 95 ºC, cooled to room temperature, and centrifuged at 3000×g for 10 min. The supernatant was poured into a pre-weighed evaporating dish to determine its soluble solids content, and the sediment was weighed (WAI, g of sediment/g flour dm). The weight of soluble solids by evaporating the supernatant overnight at 110ºC (WSI, g of soluble solids/100 g flour dm) and SP (g of sediment/g insoluble solids flour dm) were calculated.

2.6 Color characteristics

Color measurements of rice flour samples were carried out using a PCE Instruments colorimeter (PCE-CSM5) in CIELAB coordinates with 10º standard observer and D65 standard illuminant; L^* indicates lightness, a^* varies from green (-) to red (+) and b* from blue (-) to yellow (+). The hue (h) and the chroma (C^*) were also obtained from the CIELAB coordinates. Color difference (ΔE) of each treated sample with respect to control was calculated with the equation:

$\Delta E = \{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\}^{1/2}$.

Each sample was measured five times.

2.7 Pasting properties

Pasting properties of starch were determined with a Kinexus Pro+ rheometer (Malvern Instruments, UK) equipped with a starch cell. Each rice flour sample (3.5 g, 14 % moisture basis) was mixed with 25 g of distilled water before loading into the starch cell. Paddle speed was 160 rpm during the test. The sample was heated following the method described by Villanueva et al. (2018). The pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BV), final viscosity (FV), and setback viscosity (SV) were recorded. The determination was carried out in duplicate.

2.8 Rheological properties of gels made from microwaved-rice flours

The rheological tests were carried out 15 min after preparing the gels (following the protocol described in Section 2.7) to allow sample relaxation. Strain sweeps were carried out from 0.1 to 1000 % strain at 1 Hz frequency. From these curves, the maximum stress (τ_{max}) beyond which the dough structure was broken (corresponding to the linear viscoelastic zone, LVR) and the stress at the cross point (G'=G'') were established. Frequency sweeps were carried out from 1 to 10 Hz in the linear viscoelastic region (LVR); the strain value chosen for the frequency sweep tests of all gels was 1 %, which was within the LVR. The frequency sweep data were fitted to power law model (Villanueva, De Lamo, Harasym, & Ronda, 2018). The values G'_1 and G''_1, and $(\tan \delta)_1$ are the coefficients obtained from the fitting to the power law and represent the elastic and viscous moduli and the loss tangent, respectively, at a frequency of 1 Hz. The *a, b* and *c* values are the exponents of the fitted potential equations and quantify the dependence of the elastic and viscous moduli and the loss tangent on the oscillation frequency. Each test was carried out in duplicate.

2.9 Gelatinization and retrogradation properties

The untreated and the microwaved-rice flours (~6 mg) with an excess of water (70 % water) were weighed in a 40 µL aluminum pan and heated from 0 to 115 ºC at 5 ºC/min in a Differential Scanning Calorimeter (DSC3, STARe-System, Mettler-Toledo, Switzerland) using an empty sealed pan as reference. The gelatinized samples were kept in the pans at 4 ± 2 °C and after 7 days they were measured again with the same method to study their retrogradation transition. The enthalpy of gelatinization and retrogradation were recorded and expressed in J/g dry matter (dm). The degree of retrogradation, DR (%), was established as the enthalpy of retrogradation divided by the enthalpy of gelatinization. All measurements were performed in duplicate.

2.10 Statistical analysis

Statistical analyses were conducted using the software Statgraphics Centurion XVII-X64 software (Bitstream, Cambridge, MN, USA). To check the statistical importance of observed variability, one-way ANOVA was applied and the least significant difference (LSD) at p-value ≤0.05 was used to separate means.

3. Results and discussion

3.1 Temperature and moisture evolution in flour during MW treatment

During MW processing the flour temperature vs. time profiles were significantly affected by MC (see **Figure 1**). The hermetic Teflon® container used in this study made it possible to keep sample MC constant throughout treatment. This fact had a significant impact on temperature profiles. As can be seen in **Figure 1**, flour temperature increased over time until it reached, in 3-4 min, a maximum value that remained constant until the end of the treatment. Both the rate of temperature increase and the maximum temperature reached depended on the MC of the flour. The final long plateau of constant temperature corresponded to a period in which the heat absorbed by the sample was equal to the heat lost by it towards the container and from it to the surroundings (Villanueva, Harasym, et al., 2018). The plateau temperatures varied with the flour MCs as follows: 99 ± 1 ºC (3%), 173 ± 4 ºC (8 %), 138 ± 1 ºC (13 %), 141 ± 3 ºC (15 %), 136 ± 3 ºC (20 %), and 114 ± 3 ºC (30 %). The rate of the temperature increase during the first MW treatment segment was determined by two simultaneous processes governed by two properties dependent on flour MC: MW absorption capacity and specific heat. The first property determines the flour's capacity to transform MW radiation into heat, while the second one controls the amount of heat the flour needs to change its temperature. Both properties increase with MC and have an opposite effect to the rising temperature profile (Villanueva, Harasym, et al., 2018). Considering how these slopes evolve with flour MC, it seems that specific heat predominated in this step. The second treatment segment (constant temperature) was controlled by the balance between the absorbed and the dissipated power. The heat transfer coefficient --which increases dramatically with the flour MC and in particular with the presence of saturated water vapor around the sample-- explains the higher power dissipated between the flour and the Teflon® container and the lower temperature of the plateau reached by the samples with higher MCs. It should be noted that the MW absorption capacity of the flour increases with MC only logarithmically (Villanueva, Harasym, et al., 2018). The sample with an MC of 3 % rose to the lowest temperature due to its very low MW absorption capacity from its low humidity (below the monolayer MC, which in rice flour is 5 - 6 %) (Abebe & Ronda, 2015). Nevertheless, the sample temperature increase to 99ºC demonstrates that even this very low MC (3%), strongly bound to dry matter, was capable of absorbing MW radiation. The sample with 8% MC was found at the opposite end of the range. It rose to the highest temperature because of its low MC (only slightly above the monolayer level); this prevented the vapor inside the Teflon® container from reaching saturation, reducing the heat transfer between the flour and the walls of the container. The very high sample-surroundings thermal resistance led to an increase in the equilibrium temperature within the sample, despite the still low MW power absorbed.

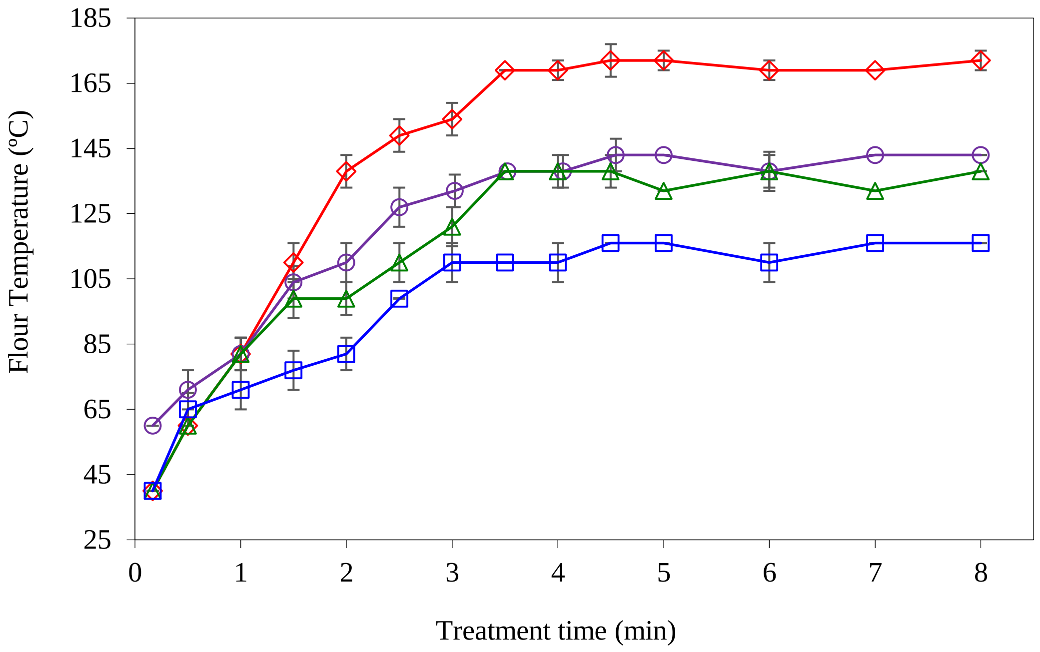


Figure 1. Evolution of temperature of the flour during the MW treatment at 8% (◊), 15% (○), 20% (Δ) and 30% (□) of moisture content.

3.2 Apparent amylose content (AAC)

The control flour used in this study could be classified as intermediate amylose flour based on its AAC, which was 16.4 ± 0.9 % (Su et al., 2018). The AAC of the samples treated at 8 % and 30 % MC were 20.7 ± 0.4 % and 18.6 ± 1.1 %, respectively, significantly higher than the native counterpart (**Table 1**). These two samples were the result of the most severe conditions (with the highest temperature or MC, respectively). The increase in AAC in DHT samples was previously reported and explained by the degradation of long-amylose chains into many short-amylose ones during dry-heating process (Lei et al., 2020). HMT may cause the thermal degradation of amylopectin exterior linear chains. Those degraded chains might become amylose-like, responsible for increasing AAC. The rupture of amylose and amylopectin interactions in the amorphous regions of starch granules would also release amylose and lead to higher AAC values (H. Liu, Lv, Peng, Shan, & Wang, 2015). Amylopectin damage presumably leads to a reduction in the amylopectin fraction and, hence, to a slight increase in AAC (Oyeyinka et al., 2019). The AAC contents of the samples treated at 3 %, 13 %, 15 %, and 20 % MC were not significantly different from native flour AAC.

Table 1. Summary of some physicochemical parameters of control (native) flour and microwaved-flours treated at different moisture content (MC).

	Control	MC 3%	MC 8%	MC 13%	MC 15%	MC 20%	MC 30%
AAC (%)	16.4 ± 0.9 a	16.6 ± 0.6 a	20.7 ± 0.4 c	16.9 ± 1.1 a	16.6 ± 0.8 a	17.3 ± 1.0 ab	18.6 ± 1.1 b
SP (g/g dm)	7.84 ± 0.40 a	8.23 ± 0.35 a	9.41 ± 0.25 cd	9.63 ± 0.24 d	9.62 ± 0.28 d	8.95 ± 0.68 bc	8.47 ± 0.28 ab
L*	85.26 ± 0.16 d	85.57 ± 0.28 d	84.54 ± 0.65 bc	85.41 ± 0.23 d	84.94 ± 0.04 cd	84.08 ± 0.08 b	82.35 ± 0.18 a
a*	0.96 ± 0.04 b	0.74 ± 0.03 a	1.6 ± 0.03 d	0.66 ± 0.04 a	0.94 ± 0.08 b	1.25 ± 0.08 c	2.08 ± 0.05 e
b*	5.41 ± 0.03 a	5.76 ± 0.01 b	8.04 ± 0.06 f	6.35 ± 0.06 d	5.98 ± 0.04 c	6.89 ± 0.11 e	8.18 ± 0.04 g
C*	5.50 ± 0.04 a	5.81 ± 0.01 b	8.20 ± 0.07 f	6.39 ± 0.05 d	6.06 ± 0.05 c	7.03 ± 0.15 e	8.44 ± 0.03 g
h	80.00 ± 0.35 c	82.65 ± 0.36 e	78.76 ± 0.15 b	84.05 ± 0.35 f	81.05 ± 0.67 d	79.68 ± 0.53 c	75.74 ± 0.39 a
ΔE	-	0.5	2.8	1.0	0.7	1.9	4.2
PT (ºC)	75.44 ± 0.10 b	74.49 ± 0.30 a	81.18 ± 0.28 d	79.54 ± 0.45 c	79.60 ± 0.56 c	81.09 ± 0.33 d	83.39 ± 0.30 e
PV (Pa·s)	5.96 ± 0.08 f	5.44 ± 0.03 e	3.81 ± 0.10 c	4.56 ± 0.01 d	4.41 ± 0.11 d	3.59 ± 0.03 b	2.51 ± 0.11 a
TV (Pa·s)	3.00 ± 0.08 de	2.90 ± 0.01 cd	2.88 ± 0.03 c	3.11 ± 0.06 e	2.95 ± 0.06 cd	2.76 ± 0.01 b	2.19 ± 0.04 a
BV (Pa·s)	2.97 ± 0.01 e	2.54 ± 0.04 d	0.93 ± 0.13 b	1.46 ± 0.05 c	1.46 ± 0.17 c	0.84 ± 0.02 b	0.32 ± 0.16 a
FV (Pa·s)	6.99 ± 0.35 c	6.86 ± 0.01 c	7.00 ± 0.11 c	7.46 ± 0.14 d	7.46 ± 0.15 d	6.27 ± 0.13 b	4.66 ± 0.04 a
SV (Pa·s)	3.99 ± 0.27 c	3.96 ± 0.01 c	4.12 ± 0.13 cd	4.36 ± 0.20 de	4.51 ± 0.09 e	3.51 ± 0.12 b	2.47 ± 0.00 a

MC 3%, MC 8%, MC 13%, MC 15%, MC 29%, MC 30%: Rice flour treated at 3, 8, 13, 15, 20 and 30% of moisture content. AAC (%): apparent amylose content; SP: swelling power; dm: dry matter. *L*, a*, b**: CIELAB color coordinates; *C**: Chroma; h: hue; ΔE: Difference of color between each sample and the control. PT: Pasting temperature; PV: Peak viscosity; TV: Trough viscosity; BV: Breakdown viscosity; SV: Setback viscosity; FV: Final viscosity. Data are the mean ± standard deviation. Values with a letter in common in the same line are not significantly different ($p<0.05$).

3.3 Hydration properties

WAC, WAI and WSI of control and microwaved rice flours are shown in **Figure 2.** SP is included in **Table 1**. The sample treated at 3 % MC did not show any significant change in these properties with regard to native flour. As demonstrated above, the energy absorbed by this sample was limited. The WACs of samples treated at 3 %, 8 %, and 13 % MC were the same as that of the control flour (≈1.3 g/g). However, WAC increased in samples treated at 15 % (+13 %), 20 % (+46 %), and 30 % MC (+46 %), under HMT conditions. These results might be attributed to disrupted hydrogen bonds between the amorphous and crystalline regions and a slight expansion of the amorphous region, improving the hydrophilic tendency of the starch molecules during HMT but not in DHT. These changes seem to be favored by a higher sample MC during thermal treatment (H. Liu et al., 2015). WAI (**Figure 2**) and SP (**Table 1**) increased in all treated samples (except 3 % MC). The highest increase in WAI was obtained for the samples treated at 8 %, 13 %, and 15 % MC (> 9 g/g *vs* 7.8 g/g for the control). In samples treated at 20 % and especially at 30 % MC, the increment was less marked. A partial pre-gelatinization caused by the high MC and temperature could explain the lower gel hydration capacity of samples treated at 20 % and 30 % MC (Iuga & Mironeasa, 2019). In addition, the short-amylose chains produced by heat hydrolysis, which easily dissociate and diffuse out of the granules during swelling (Kyung et al., 2018), would decrease sediment weight. WSI increased significantly only in samples treated at 8 % and 30 % MC, from 1.05 % (control flour) to 2.63 and 2.91 %, respectively. The increase in solubility may be a consequence of shrinkage and/or disintegration of granules, weakening amylose-amylopectin bonds and increasing amylose-water interactions (Dudu, Li, Oyedeji, Oyeyinka, & Ma, 2019).

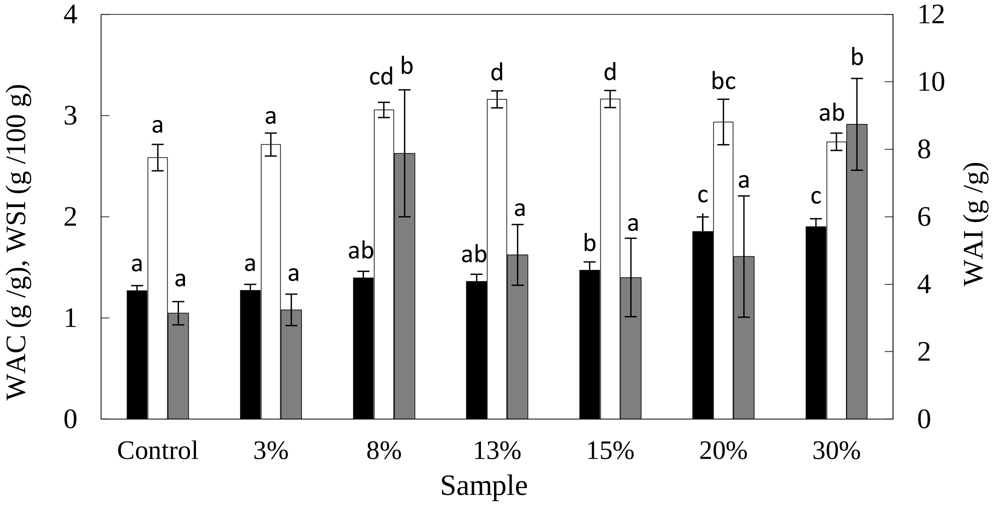


Figure 2. Hydration properties of MW treated-rice flours. WAC (water absorption capacity, g water/g flour dry matter) (black) and WSI (water solubility index, g soluble solids/100g flour dry matter) (grey) are represented on the left Y-axis and WAI (water absorption index, g sediment/g flour dry matter) (white) is represented on the right Y-axis. The error bars represent the standard deviation.

Most of the literature suggests that SP and solubility are lowered in HMT-treated starches (Hoover, 2010), which is the opposite to what was found in this rice flour study. This might be related to the interaction of starch with other components in the flour, mainly proteins and lipids (Vela et al., 2021). It has been reported that the severity of HMT-derived changes depends on temperature, and is related to structural reorganization, water restriction, and amylose-lipid, amylose-protein, and amylose-amylopectin interactions (Iuga & Mironeasa, 2019). Lei et al. (2020) also found that heating temperature caused an increase in the solubility of DHT-starch, especially when the temperature was higher than 150 °C, as was our case.

3.4 Color characteristics

The color values of the control rice flour (native) and modified samples are shown in **Table 1**. In general, the heat treatment caused a slight, although significant, decrease of lightness (L* values), indicating that the color of the treated samples (except at 3 %, 13 %, and 15 % MC, which remained unchanged) became darker, mainly at 30 % MC. Flour color is important in bakery products, mainly with regard to bread crumb color; therefore, the darkening effect could be positive in the case of white rice flour products, implying that they are whole-grain or more similar to traditional wheat bread (Rufian-Henares, 2009). The hue (h) of treated samples varied significantly with respect to the control, but in different ways depending on the treated sample MC. It changed to more reddish in samples treated at 8 % and 30 % MC, and became more yellowish at 3 %, 13 %, and 15 % MC. Chroma (C*) increased slightly in all treated samples, indicating that the treatment caused more vivid colors. A significant decrease of lightness and increase of b* coordinate were also reported for DHT-modified rice starches by Su et al. (2018). The analysis of the differences of color between each modified sample and the control, ΔE, (**Table 1**) allows to conclude that the treatments did not markedly modify rice flour color, given that ΔE was always below 5, indicating that the color differences could not be visibly differentiated (García-Viguera & Zafrilla, 2001). The low protein content of rice flour may explain the low occurrence of the Maillard reaction (MR) during MW treatment. MR and caramelization are the two reactions involved in generating color during heat treatment. Both reactions depend on temperature and water activity (Rufian-Henares et al., 2009). The formation of reducing sugars during the heat treatment (Rufian-Henares et al., 2009) was probably favored by higher MC in the flour during MW treatment, and could explain the darker color of the sample treated at 30 % MC.

3.5 Pasting properties

Typical pasting profiles of the native and the MW-treated rice flours are illustrated in **Figure 3.** The corresponding pasting data are given in **Table 1**. Non-important differences were observed between the sample treated at 3 % MC and the control flour. The most relevant change observed in this sample was a decrease in PT (-1 ºC); this was opposite what happened in the other treated samples, where PT increased from 4 ºC to 8 ºC with respect to the control flour. DHT would break intra- or inter-molecular hydrogen bonds of starches, which would facilitate the swelling of starch granules (Lei et al., 2020; Li et al., 2013). A higher PT indicates greater resistance to swelling and rupture. The maximum increases in PT were observed in samples treated at an MC of 8 % (+5.8 ºC), under DHT conditions, and at 20 % (+5.7 ºC) and 30 % (+8 ºC), under HMT conditions. The treatments decreased the PV of all modified samples, with the maximum decreases found in the same MC samples: 8 % (-37 %) <20 % (-40 %) <30 % (-58 %). The treatments at 13 % and 15 % barely showed any differentiations in PV and the remaining pasting properties. According to Li et al. (2013), excessive heat could separate glycosidic linkages and disrupt hydrogen bonds, which could decrease viscosity. Lei et al. (2020) also concluded that DHT of starches at temperatures above 170 ºC decreased the viscometric profiles, probably due to a partial thermal degradation of amylose and amylopectin molecular and crystalline structure. This could explain why the sample treated at 8 % MC, which reached 173 ºC, presented a lower PV than those at 13 % and 15 % MC. In the case of HMT, other authors also reported a decrease in PV and BV dependent on treatment conditions (Iuga & Mironeasa, 2019). BV decreased in all treated samples with respect to the control, with the lowest values observed for the samples treated at MCs of 8 % (-69 %), 20 % (-72 %), and 30 % (-89 %). The low BV of treated flour indicates a higher stability of the swollen granules versus shear and heating (Villanueva, Harasym, et al., 2018).

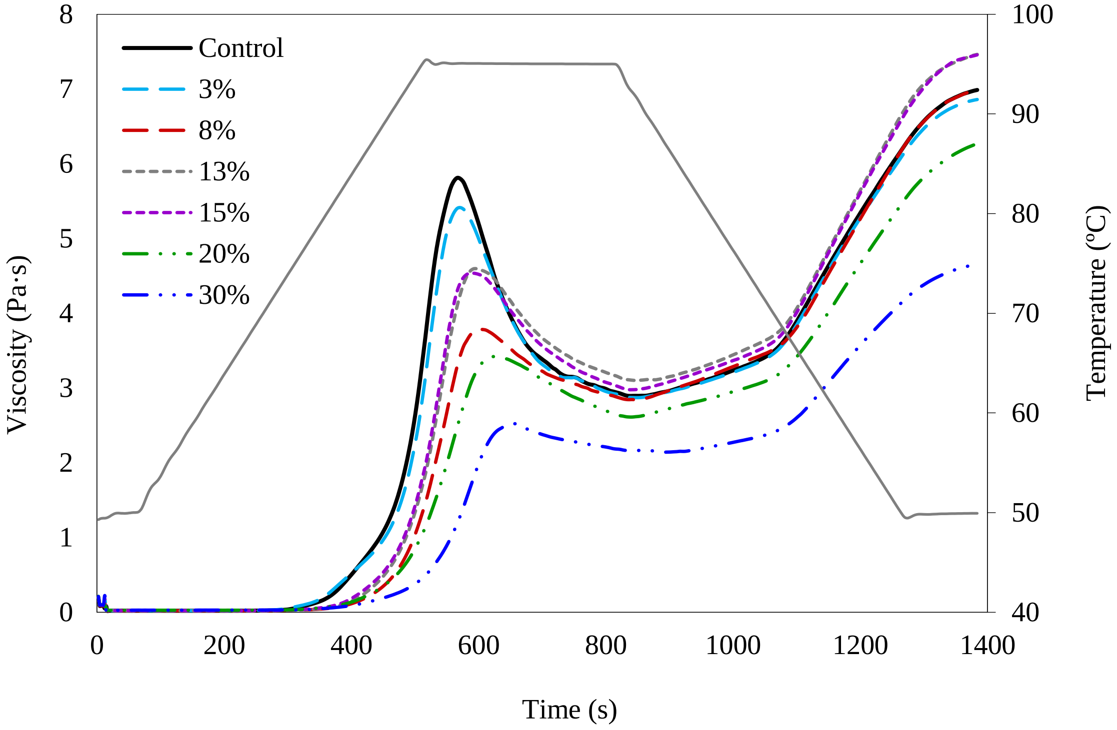


Figure 3. Pasting profiles of MW treated-rice flours. Continuous grey line represents the temperature (right Y-axis).

In this study flours with low swelling capacity exhibited less decrease in viscosity after reaching the maximum value. SV is usually associated with the potential for retrogradation in starches, and lowering it means that there is less chance for retrogradation (Yang et al., 2017). The SV values of 13 % and 15 % MC samples increased by 9 % and 13 % with respect to the control, while the samples treated at 20 % and 30 % MC decreased by 12 % and 38 %, respectively. The drop in the FV of the sample treated at 30 % MC, which was 33 % lower than the control sample, suggested that short-term (amylose) retrogradation could be decreased by the MW treatment under these conditions. The results obtained revealed that the pasting parameters did not show a regular evolution with MC as reported for other starch systems (Braşoveanu & Nemţanu, 2014).

3.6 Rheological properties of gels obtained from treated flours

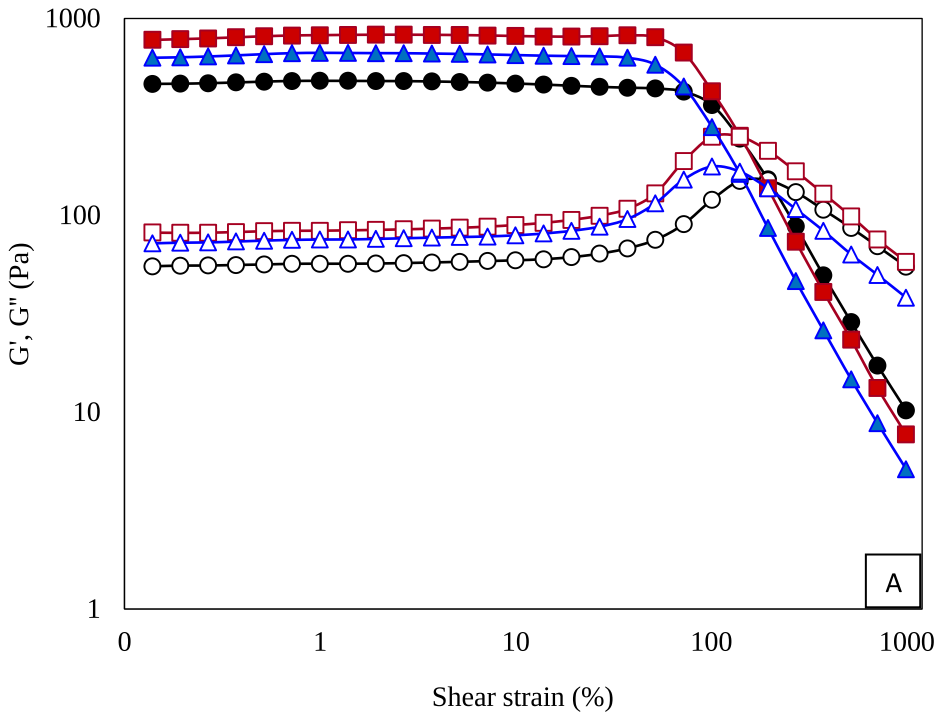
The rheological properties of gels made from MW-treated rice flour were determined and compared to those of the gel made from the control (untreated) flour using dynamic oscillatory tests. **Table 2** presents the parameters obtained from fitting the power law model to the frequency sweep data, the maximum stress (τ_{max}) within the LVR, as well as the stress at which the gels passed from solid-like to viscous-like behavior (G'= G'' and tan δ= 1**)**.

The oscillatory test revealed that MW treatment affected the rice flour gel viscoelastic moduli regardless the MC of the flour during the treatment (**Figure 4**). The gel made from rice flour treated at 3 % MC did not present significant differences from the control rice flour, except for the τ_{max}, which increased from 315 Pa to 435 Pa. This indicates that at least the resistance of the gel to disruption was significantly improved as a result of the treatment. In the rest of samples, all viscoelastic properties showed significant differences with respect to the control (**Table 2**). The most marked effect was observed on the sample treated at 8 % MC, closely followed by that treated at 20 % MC. Storage (G'_1) and loss (G''_1) moduli increased markedly with MW treatment with regard to the native flour following the order (the increase in G'_1 and G''_1, respectively, with regard to the control is shown in parentheses): 8 % (+66 %, +46 %) ≈ 20 % (+61 %, +44 %) > 30 % (+35 %, +32 %) ≈ 15 % (+44 %, +26 %) ≈ 13 % (+36 %, +21 %). The elastic modulus always increased more than the viscous one, explaining the decrease in the loss tangent (G''/G') with MW treatment. The values of G'_1 were only slightly dependent on frequency, as the low "a" exponents (0.05-0.08) denote, significantly lower in gels made from microwaved-flours. This confirms that their structure was more stable, explained by the significantly higher elastic modulus of treated samples. The rate of increase of G" with frequency ("b" exponent range: 0.26-0.31) was five times that of G', which indicates that the loss tangent (G''/G') increased significantly with frequency (with "c" values in the 0.20-0.23 range). The $(\tan\delta)_1$ decreased slightly in treated samples, from 0.12 to 0.10, which means that the MW treatment yielded gels with more pronounced solid-like behavior. The τ_{max} and cross-over point showed their maximum values for the gels obtained from rice flour treated at 8 % MC (60 % higher than the control gel).

Table 2. Effect of MW treatment on the rheological properties and retrogradation degree in seven days of rice flour gels made from native (control) flour and microwaved-flours treated at different moisture content (MC).

	Control	**MC 3%**	**MC 8%**	**MC 13%**	**MC 15%**	**MC 20%**	**MC 30%**
G'_1 (Pa)	470 ± 4 a	452 ± 1 a	782 ± 16 c	638 ± 10 b	676 ± 47 b	758 ± 33 c	635 ± 39 b
a	0.080 ± 0.002 d	0.075 ± 0.012 cd	0.050 ± 0.001 a	0.065 ± 0.012 bc	0.055 ± 0.010 ab	0.060 ± 0.001 ab	0.060 ± 0.001 ab
R^2	0.9987	0.9956	0.9990	0.9995	0.9986	0.9997	0.9995
G''_1 (Pa)	57 ± 2 a	56 ± 1 a	83 ± 1 d	69 ± 3 b	72 ± 1 bc	82 ± 3 d	75 ± 2 c
b	0.310 ± 0.001 c	0.305 ± 0.010 c	0.265 ± 0.010 ab	0.280 ± 0.002 b	0.271 ± 0.010 ab	0.255 ± 0.011 a	0.275 ± 0.010 b
R^2	0.9995	0.9987	0.9998	0.9996	0.9982	0.9975	0.9985
$(\tan \delta)_1$	0.120 ± 0.002 c	0.120 ± 0.001 c	0.110 ± 0.001 ab	0.110 ± 0.003 ab	0.105 ± 0.010 a	0.110 ± 0.002 ab	0.115 ± 0.012 bc
c	0.230 ± 0.001 b	0.230 ± 0.002 b	0.210 ± 0.002 a	0.215 ± 0.010 ab	0.210 ± 0.002 a	0.200 ± 0.013 a	0.215 ± 0.011 ab
$\tau_{\text{máx}}$ (Pa)	315 ± 14 a	435 ± 4 d	506 ± 9 e	443 ± 10 d	404 ± 6 c	363 ± 4 b	310 ± 12 a
Cross over (Pa)	149 ± 8 a	155 ± 2 ab	250 ± 1 e	201 ± 3 c	220 ± 3 d	206 ± 5 c	167 ± 9 b
$\triangle H_{gel}$ (J/g dm)	9.05 ± 0.27 c	8.77 ± 0.26 c	8.27 ± 0.30 c	8.43 ± 0.34 c	8.35 ± 0.10 c	4.63 ± 0.39 b	3.18 ± 0.22 a
DR (%)	64 ± 4 a	60 ± 1 a	74 ± 1 a	68 ± 6 a	63 ± 5 a	124 ± 12 b	167 ± 13 c

MC 3%, MC 8%, MC 13%, MC 15%, MC 29%, MC 30%: Rice flour treated at 3, 8, 13, 15, 20 and 30% of moisture content. The power law model was fitted to experimental results from frequency sweeps. $G' = G'_1 \cdot \omega^a$; $G'' = G''_1 \cdot \omega^b$; $\tan \delta = (\tan \delta)_1 \cdot \cdot \omega^c$). $(\tan \delta)_1$ was obtained from the quotient $G''_{\omega 1}/ G'_{\omega 1}$ and *c* from *b-a*.. G_1', G_1'' and $(\tan \delta)_1$: represent the elastic and viscous moduli and the loss tangent at a frequency of 1 Hz. The a, b and c exponents: quantify the dependence degree of dynamic moduli and the loss tangent with the oscillation frequency. R^2: Fitting regression coefficient. τ_{max}: maximum stress that samples can tolerate in the LVR; DR (%) $=100 \cdot \Delta Hret/\Delta Hgel$. ΔHgel, ΔHret: melting enthalpy of crystallized amylopectin during the gelatinization and retrogradation scans respectively; dm: dry matter. Data are the mean ± standard deviation (n = 2). Values with a letter in common in the same line are not significantly different ($p<0.05$).



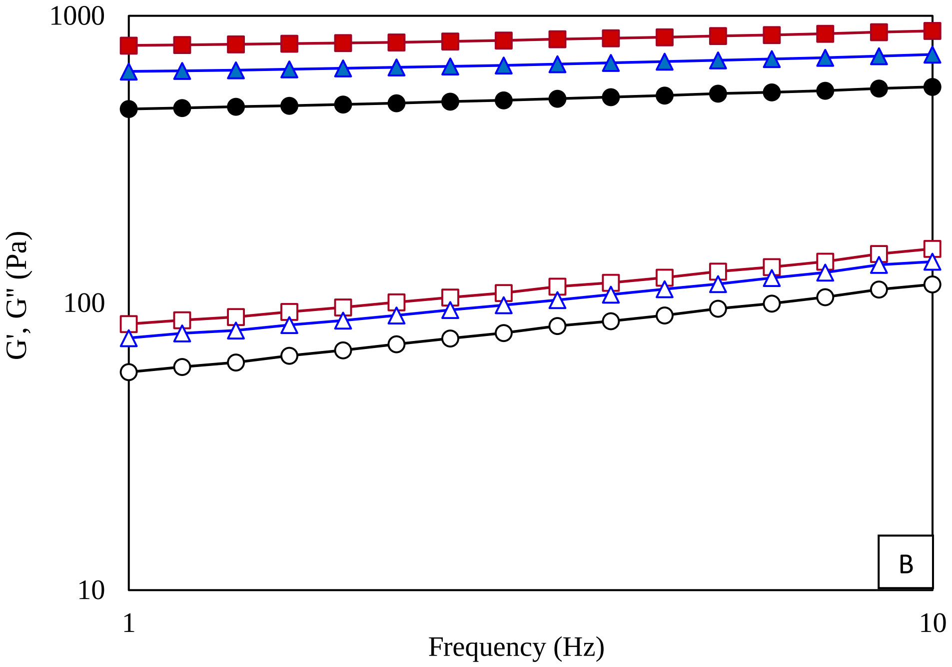


Figure 4. Strain sweeps (A) and frequency sweeps (B) of gels prepared with control rice flour (●○), 8% (■□) and 30% (▲,△) MW-treated-rice flours. G’ represented by full symbols, G’’ represented by empty symbols.

Both values decreased with increasing MC of the flour during MW treatment. This confirms that DHT leads to the most stable and consistent and the strongest gel structures. Similar results were obtained by other authors when DHT was applied to normal and waxy corn starches (Lei et al., 2020; Qiu et al., 2015), when different starches were treated with traditional HMT processes (Hoover, 2010), or when rice starch was treated by MW radiation (Villanueva, De Lamo, Harasym, & Ronda, 2018). Comparing both conditions, we can conclude that the rheological properties of the gel were more affected by DHT than by HMT and that, within the latter, the 20 % MC generally led to greater changes than the 30 %.

3.7 Gelatinization and retrogradation properties

The gelatinization enthalpy of native and microwaved-samples is shown in **Table 2**. A slight, not significant, decrease in the gelatinization enthalpy was detected in samples treated from 3 % to 15 % MC. However, in samples treated at 20 % and 30 % MC the gelatinization enthalpy decreased 54 % and 65 %, respectively, in reference to that of native flour. This denotes a partial gelatinization of these two flour samples during the MW treatment.

Retrogradation could be categorized into two processes: the gelation of amylose solubilized during gelatinization, which happens in a short period of time (minutes-hours); and the recrystallization of amylopectin, which takes significantly longer (7-10 days) (Ploypetchara, Suwannaporn, Pechyen, & Gohtani, 2015). Amylopectin retrogradation can be considered as a time- and temperature-dependent polymer recrystallization process. The melting enthalpies of retrograded amylopectin in treated rice flours after 7 days of storage at 4ºC were recorded and compared to that obtained in the control (untreated) flour. No significant differences were observed in retrogradation enthalpy, which was ≈5.7 J/g dm in all samples, which denotes that the ability of amylopectin to re-associate after gelatinization was not altered by the MW treatment. However, the degree of retrogradation (DR) changed in some of them (**Table 2)**. For the control flour and flours treated at 3 %, 8 %, 13 %, and 15 %, the DR was 60-74 %, with no significant differences among them. However, in the 20 % and 30 % MC samples the DR values were 124 % and 167 %, respectively, as result of the partial gelatinization caused by the high temperature and moisture level during the treatment of these samples.

4. Conclusions

MW-treated freeze-dried rice flour (3 % MC) showed no change from native flour in techno-functional properties. However, the flour treated at 8 % MC showed important changes in the hydration and pasting properties studied and led to the most consistent and resistant gels. This small amount of water (slightly above the monolayer MC) was sufficient to absorb MW radiation and to exert a plasticizing effect for the molecular rearrangements of starch, responsible for these changes. The lowest peak and breakdown viscosities and the highest pasting temperatures were found in the samples treated at 30 %, 20 %, and 8 % MC. DSC revealed that the flours treated at 20 % and 30 % MC were partially pregelatinized, while the low MC of the remaining samples prevented granular disruption. MW treatment increased the WAC and SP of all treated flours. It also increased the solubility of flours treated at 8 % and 30 % MC. The significantly higher AAC of these two samples with respect to that of the control flour could explain these results and confirm the highest severity of the treatment under these two conditions (the highest temperature and the highest MC).

The study shows that the MC of the flour during MW treatment makes it possible to modulate the techno-functional properties of the MW-modified flour and the rheological and thermal characteristics of the gels obtained from them, being the MC of 8 % the most effective in these changes. These MW-treated rice flours can be used in the production of food products with tailor-made functional properties suitable for the celiac population.

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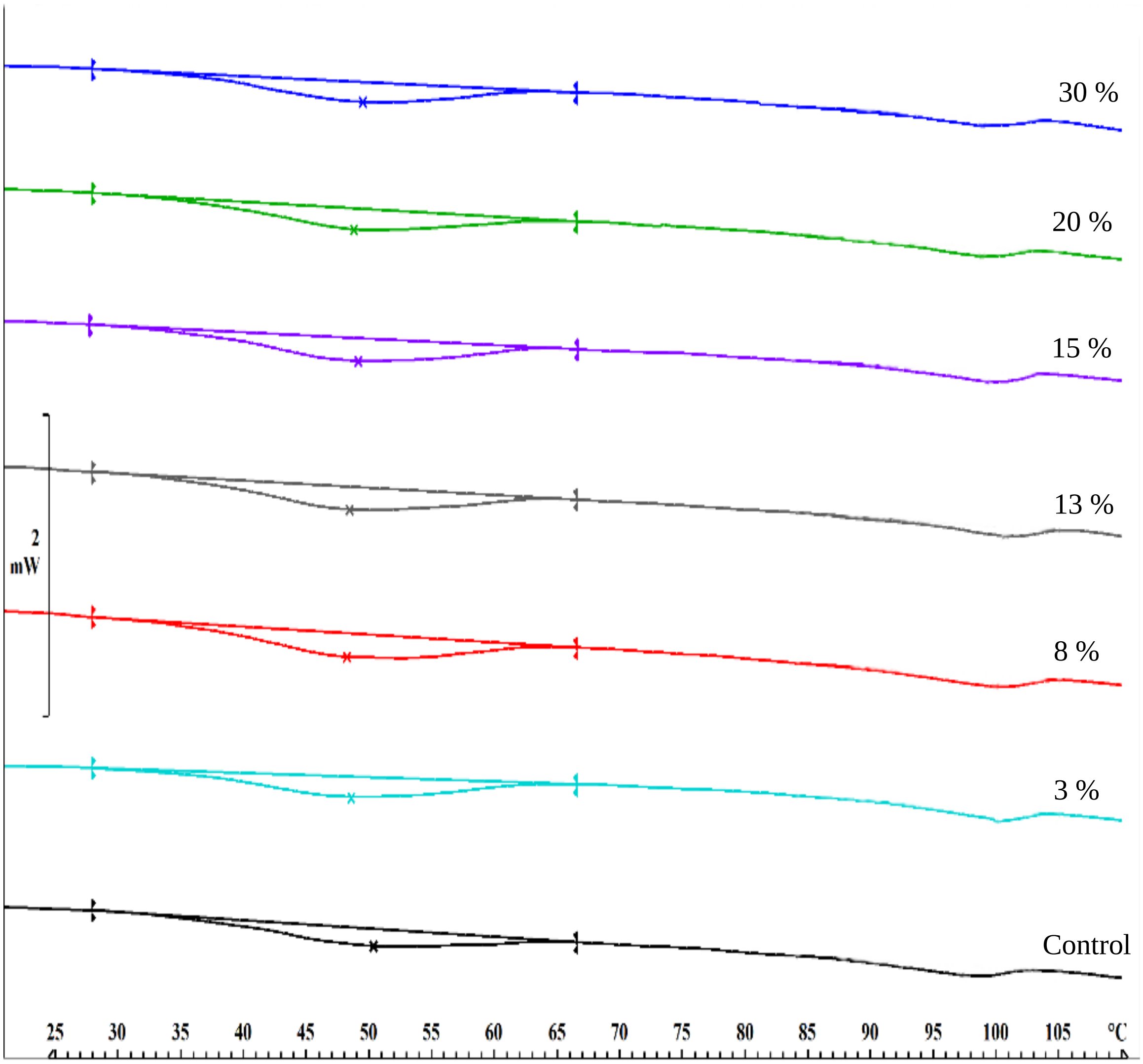

Supplementary Figure 1: Thermograms of retrograded control flour and microwaved treated rice flours after 7 days of storage at 4 ± 2ºC.