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Effect of annealing and α-amylase extract on the rheological properties, syneresis, and water holding capacity of different starches

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Abstract

The objective of this study was to determine the impact of annealing and enzyme extract on the gel properties of chickpea (C.P), corn (C.S), Turkish bean (T.B), sweet potato (S.P.S), and wheat starches (W.S). Starches were annealed at different temperatures and times in excess water with or without germinated sorghum extract (GSE). The concentration of α-amylase in the GSE was 5 mg/10 mL. Dynamic rheological properties, freeze-thaw stability and water holding capacity (WHC) were investigated. The dynamic rheological parameters of the native or GSE-treated starches varied significantly ($p < 0.05$), while the G` of some starches were frequency-independent others exhibited sharp increase in G` at low frequencies. Unlike T.B and S.P.S, the G` of the native C.P and C.S starches was significantly ($p < 0.05$) reduced by annealing, whereas GSE-treatment reduced G` of all gels regardless of annealing temperature or time. Starch gels demonstrated significant ($p<0.05$) reduction in freeze thaw stability and increase in water holding capacity after annealing and GSE treatment.

Keywords: annealing; α-amylase; rheology; freeze-thaw; water holding.

Practical Application: The scope of this work was focused on shedding some light on the use of germinated sorghum extract in the starch industry as a replacement for pure α–amylase enzyme preparation in starch modification. The advantage of using sorghum is its low price and availability. In addition, germinated sorghum is a simple, save, and inexpensive process and is a good source of α–amylase. This process can be achieved by a very low initial cost.

1 Introduction

Starch is primarily two major molecules, amylose (AM) and amylopectin (AP) build of D-glucose. Amylose is a linear polymer of glucose linked by α-1-4 glycosidic bond, while amylopectin is branched α-1-6 glycosidic bond in addition to α-1-4 glycosidic. Native starch utilization is limited because of gel instability "retrogradation" during storage. This property can be addressed by physical, chemical or enzymatic modifications. The enzymatic digestion of starch is focused on measuring starch susceptibility to different enzymatic attack. It is critical to maximize the process and reduce the cost of bioconversion (use of enzymes) of starch to glucose, ingredients or fuel (Lopez-Rubio et al., 2008). Endo-acting α-amylase from different sources hydrolyses α-(1-4) bonds in a random manner, thereby initiates starch granule attack and reduces its molecular weight (amylose and amylopectin). Earlier literature reports have shown that the action of α-amylase kinetics on starches from different botanical origin is diverse and the outcome of the degradation is different products (Sarikaya et al., 2000; O'Brien & Wang, 2008). The rate of hydrolysis of starch by α-amylase is complex and depends on the granule size, integrity, crystallinity, porosity, amylose content, and granule structure (Copeland et al., 2009).

At higher temperature, hydrogen bonding between starch molecules within the granule is broken and consequently, leached amylose forms a three-dimensional network mass. Upon gelatinization under specified conditions, starch forms viscous mass consisting of a continuous phase of solubilized amylose and/or amylopectin and one discontinuous phase of the remaining swollen un-gelatinized starch granules (Sarker et al., 2013; Ambigaipalan et al., 2011; Alcázar-Alay & Meireles, 2015). The length, amylose and amylopectin ratio and the degree of branching define the starch granule size, structure and specific function in each botanical group. Other characteristics associated with the granule surface-smoothness and presence of phosphate groups can influence the properties and application of the paste, as well (Smith, 2001).

The textural state of the final product with respect to structure and appearance, is dependent on the intensity of the processing operations such as temperature and mixing (Valetudie et al., 1999). Stress and strain by and large are used to characterize rheological properties of foods using different mathematical models such as power law with and without yield stress (Marcotte et al., 2001; Lawal et al., 2011). The specific adjustment of rheological properties is critical for food production, processing and optimization of stability (Yoo et al., 2005). The dynamic rheology of starch includes parameters such as storage dynamic modulus (G`), the loss modulus (G``) and the ratio of G``/G` which is defined as tan δ. Starches, in general, exhibit low tan δ which indicates elastic gel (Singh & Kaur, 2017). Oh et al. (2018) demonstrated

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how native corn starch from different sources exhibited a wide range of G` as a function of temperatures. Ideal elastic materials exhibit G' independent of frequency and much higher than G`` (G`> G``) (Singh & Kaur, 2017). Starch steady shear rheology can be investigated during gelatinization or on pastes in terms of flow, viscoelasticity, mechanical spectra, creep, and gel strength. Power law and Herschelle-Bulkely models are commonly used to illustrate the flow behavior of starches. These models provide information on the flow behavior index (n) and the consistency coefficient (K) and yield stress (so) of starch gel. Starch slurry heated to about 90˚C are reported to exhibit shear-thinning (pseudoplastic) behavior with flow behavior index (n) by far less than 1.0 (Lawal et al., 2011). In steady shear test of starch gel, high power law K value indicates high structural strength resulting in a less thixotropic behavior (Wani et al., 2013). The n value of the power law indicates the extent of shear thinning behavior as it deviates below 1.0. Yield stress is the minimum stress required to initiate flow, which is indicative of entanglement or other interactive forces between molecules in a system that must be neutralized before flow can occur at a considerable rate. Successive freeze-thaw cycles intensify phase separation and results in the formation of larger ice crystals. Upon thawing, the released water can easily separate from the gel network (syneresis) (Yuan & Thompson, 1998; Eliasson & Kim, 1992).

Although the activity of other enzymes increases during seeds germination, the activity of α-amylase only is the focus of this work. The objectives of this work were to estimate the concentration of α–amylase in the germinated sorghum extract (GSE) and determine the effect of annealing and crude GSE on the rheological, water holding capacity, and freeze-thaw stability of legume, tuber and cereal starches under different annealing conditions. The economical outcome of this work will be to introduce the crude (un-purified) α-amylase extract for use in the starch industry for starch modification or syrup production.

2 Materials and methods

2.1 Materials

Starches from chickpea (C.P), Turkish beans (T.B), sweet potato (S.P.S) and wheat starch (W.S) were isolated from raw materials purchased from the local market (Riyadh, Saudi Arabia), whereas corn starch (C.S) was donated by ARASCO Company (Riyadh, Saudi Arabia). The starch isolation methods were detailed in a previous publication by the same authors which is under review (Alqah et al., 2020). Aspergillus fungal α-amylase (EC3.2.1.1) was purchased from Sigma Aldrich (St Louis, Missouri, USA). The pure α-amylase was used to estimate the enzymatic activity of the germinated sorghum seed extract used in this study. The centrifugation step of starch isolation was done using Beckman Centrifuge (Beckman JXN, Brea, CA USA). The amylose content of the tested starches was determined using the method detailed by (Alqah et al., 2020)

2.2 Starch modification

Sorghum seeds were germinated at 24 °C, 25% moisture for 4 days, air dried, ground and 10 g were added to 40 mL distilled water, stirred for 15 min and filtered through Whitman 40 and centrifuged for 10.0 minutes at 2000×g. The supernatant was considered germinated sorghum extract (GSE). Fresh extract was prepared daily. Starch was modified using the GSE as follows: Starch (30 g) was placed in glass jar and 270 mL of distilled water was added to obtain a ratio of: starch: water, 1: 9 (w / v) and 0.10 mL or 1.0 mL of GSE were added to the starch slurry. The control was considered as annealed starch slurry without GSE. Slurry was stirred and annealed in water bath at 40, 50 and 60°C for 30 or 60 min, centrifuged to remove the residual enzyme. Centrifugation step was repeat three times, using fresh distilled water. After washing, the starch was dried by adding 100 mL of acetone then air dried. The dried starch was passed through a 250 µm wire sieve and stored at -20 °C for further analysis. The α-amylase activity of GSE was estimated indirectly by treating corn starch with specific concentration of pure α-amylase and determine its pasting properties after treatment. Therefore, 50 mg/100 mL α-amylase (EC3.2.1.1) solution was prepared and from that, a set of samples were prepared by adding 0.1, 0.2, 0.3, 0.4, or 0.5 mL were added to 2.8 g of corn starch. Similarly, the same volume of GSE was added to 2.8 g of corn starch. The pasting properties of both corn starch slurries with pure α–amylase or GSE samples were determined using RVA as described by Alqah et al., (2020). The pasting properties profiles of the starches in each pure enzyme or GSE concentration were matched to determine the enzyme activity corresponding to the specific volume added. Based on that, the α-amylase concentration in the GSE was found to be 5 mg/10 mL.

2.3 Water Holding Capacity (WHC) (g/g)

The water holding capacity (WHC) was measured according to the method described by Berton et al. (2002) which is based on the AACC (American Association of Cereal Chemists, 2000) method no. 88-04 with some modification. The starch used for this test was treated with germinated sorghum extract at 40, 50, and 60˚C, washed with distilled water and air dried. Starch sample (1.0 g) (W0) was suspended in 5 mL of water and vortexed for 10 s. The sample was left at room temperature (25±2°C) for 30 min, then centrifuged at 2000 x g for 10 min, and the sediment was weighed (W1). The WHC was calculated as g of the water absorbed per g of starch according to the following relationship: WHC (g/g) = W1- W0 / W0

Where W0 is the initial weight (g) of a starch sample before treatment and W1 is the final weight (g) of a starch sample after treatment. The results are the mean values of three replications.

2.4 Freeze-thaw stability of starch gels

The freeze-thaw stability was determined as described by Alamri et al. (2013), with some modifications. After dynamic rheological measurements, the formed starch gel 8g (M_i) was stored in centrifuge tubes at -20°C. The tubes were removed on the fourth day and placed in a bath for 30 min at 50°C, centrifuged at 2000 x g for 15 min. The separated water from gels was measured (M_f). The tubes were restored at -20°C for another 4 days for another thaw cycle. Syneresis was calculated according to the following equation: %syneresis = M_i-M_f / M_i x 100

2.5 Dynamic rheological properties of starch gels

The dynamic rheological testing was done using DHR Hybrid Rheometer (TA Instruments, New Castile, PA), which was programmed to collect dynamic rheological properties under varying oscillations. Starch sample (2.8 g, 14% M.C) was placed in special aluminum cell and the total weight of 28 g was completed by adding distilled water. The operational gap between the peddle in the inner perimeter aluminum pan was 5500 µm throughout the experimental. The experimental conditions of the pasting properties were: Peddle rotational speed was 132 (rad/s), sample was held at 50°C for 2 min, ramped to 95°C @10°C/ min, held at 95°C for 4 min, cooled down to 50°C @10°C/ min, held at 50°C for 2 min. All pasting parameters were recorded from the curve. After pasting step, the gel (in the starch cell) was further investigated for dynamic rheological properties. The range of the linear viscoelastic range (LVR) was determined by running strain-sweep experiment to make certain that all measurements were done within the LVR. The LVR test was attained by increasing the strain from 0.1 to 50.0% at constant frequency of 0.1 Hz (0.628 rad/s). Constant strain (5%) was used, which was within the linear viscoelastic region (LVR), and the dynamic properties were recorded at 60°C between 0.1-100 (rad/s) angular frequencies. Hence, the frequency sweep was set between 0.1 to 100 (rad/s) with constant strain of 5.0%. This indicates that the measured parameters are independent of the applied shear strains. Measurements were replicated at least twice using fresh samples per test and the relative errors were about ±10%. Rheology Advantage Data Analysis software (Version 5.7.0) provided by TA instrument was used to analyse the data. The frequency between 0.1 to 100 (rad/s) used here is usually used for most biomaterials to determine G`, G``, and η*. The storage dynamic modulus (G`) is a measure of the energy stored in the material and recovered from it per cycle, while the loss modulus (G``) is a measure of the energy dissipated (lost) per cycle of sinusoidal deformation. The ratio of the energy lost to the energy stored for each cycle is defined by tan δ.

3 Results and discussion

3.1 Water Holding Capacity (WHC) (g/g)

In a previous publication by Alqah et al. (2020) we reported the % amylose content of wheat (W.S.), chickpea (C.P.), sweet potato (S.P.S.), Turkish beans (T.B.), and corn starch (C.S.) was 25.0% ± 0.07%, 24.0% ± 0.09%, 22.6% ± 0.06%, 20.9% ± 0.06%, and 20.4% ± 0.08%, respectively. The water holding capacity (WHC) of annealed, native and GSE treated starches is presented in Table 1.

Annealing appeared to decrease WHC of the tested starches by 4-5%, except for TB and SPS since their WHC increased by 4 and 2.4%, respectively. The WHC of native starches rank from the highest to the lowest as: C.P>T.B>W.S>S.P.S>C.S, whereas annealed samples were C.P>T.B>S.P.S>C.S>W.S. According

Table 1. The effect of germinated sorghum extract (GSE) on the water holding capacity of starches.

		40˚C				
		C. P	C.S	T.B	W.S	S.P.S
	Native	$1.20^{a} \pm 0.03$	$0.74^{d} \pm 0.07$	$0.97^{b} \pm 0.07$	$0.88^{c} \pm 0.05$	$0.83^{c} \pm 0.05$
30 min	No GSE	$1.15^{a} \pm 0.1$	$0.70^{d} \pm 0.06$	$1.01^{b} \pm 0.08$	$0.68^{d} \pm 0.03$	$0.85^{c} \pm 0.04$
	0.1 mL	$1.16^{b} \pm 0.1$	$0.83^{d} \pm 0.08$	$1.26^{a} \pm 0.1$	$0.78^{d} \pm 0.01$	$0.93^{c} \pm 0.09$
	1.0 mL	$1.51^{a} \pm 0.1$	$0.97^{c} \pm 0.14$	$1.41^{ab} \pm 0.5$	$0.83^{d} \pm 0.10$	$0.95^{c} \pm 0.1$
60 min	No GSE	$1.15^{a} \pm 0.7$	$0.79^{c} \pm 0.03$	$0.97^{b} \pm 0.02$	$0.60^{d} \pm 0.05$	$0.79^{c} \pm 0.03$
	0.1 mL	$1.28^{a} \pm 0.1$	$0.88^{c} \pm 0.11$	$1.10^{b} \pm 0.09$	$0.77^{d} \pm 0.11$	$0.80^{c} \pm 0.05$
	1.0 mL	$1.53^{a} \pm 0.2$	$0.91^{c} \pm 0.2$	$1.18^{b} \pm 0.08$	$0.80^{d} \pm 0.13$	$0.81^{d} \pm 0.02$
		50 °C				
	Native	1.14 ± 0.05	0.88 ± 0.08	0.79 ± 0.02	1.10 ± 0.05	0.87 ± 0.03
30 min	No GSE	$1.10^{a} \pm 0.07$	$0.79^{d} \pm 0.07$	$0.86^{c} \pm 0.04$	$0.90^{b} \pm 0.01$	$0.85^{c} \pm 0.07$
	0.1 mL	$1.24^{b} \pm 0.01$	$0.88^{d} \pm 0.05$	$1.03^{c} \pm 0.02$	$1.32^{a} \pm 0.1$	$0.88^{d} \pm 0.09$
	1.0 mL	$1.33^{b} \pm 0.04$	$0.93^{d} \pm 0.03$	$1.13^{c} \pm 0.04$	$1.51^{a} \pm 0.12$	$0.93^{d} \pm 0.12$
60 min	No GSE	$1.11^{a} \pm 0.06$	$0.86^{c} \pm 0.08$	$1.06^{b} \pm 0.09$	$0.90^{c} \pm 0.05$	$0.72^{d} \pm 0.05$
	0.1 mL	$1.25^{a} \pm 0.01$	$0.91^{d} \pm 0.01$	$1.09^{bc} \pm 0.10$	$1.17^{b} \pm 0.09$	$0.81^{de} \pm 0.04$
	1.0 mL	$1.57^{a} \pm 0.03$	$0.97^{cd} \pm 0.04$	$1.08^{c} \pm 0.11$	$1.29^{b} \pm 0.13$	$0.82^{d} \pm 0.06$
		60 °C				
	Native	$1.22^{a} \pm 0.06$	$0.87^{d} \pm 0.01$	$0.94^{b} \pm 0.04$	Gel	$0.91^{c} \pm 0.02$
30 min	No GSE	$1.17^{a} \pm 0.0$	$0.83^{c} \pm 0.08$	$0.98^{b} \pm 0.09$	Gel	$0.94^{b} \pm 0.04$
	0.1 mL	$1.40^{a} \pm 0.1$	$0.99^{c} \pm 0.11$	$1.01^{b} \pm 0.12$	Gel	$0.78^{d} \pm 0.05$
	1.0 mL	$1.69^{a} \pm 0.1$	$1.04^{c} \pm 0.13$	$1.13^{b} \pm 0.01$	Gel	$0.73^{d} \pm 0.03$
60 min	No GSE	$1.27^{a} \pm 0.08$	$1.04^{b} \pm 0.08$	$1.02^{b} \pm 0.06$	Gel	$0.81^{c} \pm 0.06$
	0.1 mL	$1.25^{a} \pm 0.1$	$0.93^{bc} \pm 0.1$	$1.04^{b} \pm 0.10$	Gel	$0.75^{d} \pm 0.02$
	1.0 mL	$1.23^{a} \pm 0.0$	$0.79^{c} \pm 0.1$	$1.20^{a} \pm 0.0\ 0$	Gel	$0.49^{d} \pm 0.08$

C.P = chickpea; C.S=corn starch; T.B=Turkish beans; W.S=wheat starch; S.P.S=sweet potato starch. Values followed by different letters within each raw are significantly different ($p \leq 0.05$); Water holding capacity reduction due to germinated sorghum extract; ± SD = Standard deviation.Gel: gelatinization of wheat starch at 60 °C.

to these ranks amylose content was not the main determining factor of the WHC, because, W.S had the highest amylose content and did not rank first. Botanical origin could be considered as a leading factor since C.P and T.B (legumes) exhibited the highest values. Schirmer et al. (2013) reported higher WHC for maize starch, with 71% amylose content, than common maize with 23% amylose, whereas the same authors reported higher WHC for waxy potato and waxy barley compared to the regular. The results presented here are not in total agreement with Schirmer et al. (2013) work, given that high amylose starches are easily penetrated by water. This was true for C.P, but not for W.S. Thereby, granule structure and a loose association between the molecules on the amorphous region of the granule are the cause for the amount of WHC rather than amylose content only. Therefore, WHC can be used to estimate granule surface permeability (porosity). Consequently, the data presented here indicate that the amorphous region of W.S granules was more compact compared to C.P, whereas C.S was the most compact due to the least amount of WHC. The data in Table 1 showed that annealing decreased the WHC of the tested starches except for S.P.S. Starch granules bind water via hydrogen bonding which indicates the amount of water that starch can hold. The differences between the WHC of the starches can be attributed to the intensity of the hydrogen bonds and the accessibility of water binding sites in the granule. Therefore, WHC of starches is dependent on granule structure, amylose content, botanical origin and type of treatment applied on the starch. Previous reports showed that WHC, swelling power, and peak viscosity are correlated, but amylose content did not appear to be a major indicator of these parameters (Lee et al., 2012). Previous reports found no correlation between starch peak viscosity and WHC of the same starches (Alamri et al., 2013). Oh et al. (2018) reported higher WHC for dry-heat treated starches compared to native starches, which is different from the wet-heat treatment applied in this study. It is apparent that dry-heat treatment increases granule porosity leading to higher WHC. Overall, C.P behavior stands out, because it did rank first with respect to WHC and second regarding the peak viscosity (Alamri et 2013) and amylose content. The ability of starch to bind and hold water is a desirable characteristic in the food industry especially when starch is used in frozen food products as stabilizers and emulsifiers because it prevents syneresis. The GSE-treated starches exhibited higher WHC, except for S.P.S and C.S treated at 60˚C for 60 min and 1.0 mL GSE. Given that α-amylase attack causes holes on the starch granule surface, which explains the increase in WHC, but the drop in WHC of S.P.S and C.S after GSE treatment could be attributed to starch granules aggregation induced by annealing temperature and time. Hence, the wet annealing used here limited the accessibility of hydration sites leading to reduction in WHC, while enzyme attack allowed for new hydration sites, which leads to higher WHC. In other words, annealing reduced the number of available hydroxyl groups through hydrogen bonds between starch chains, thereby, presented a smaller capacity of water retention. Starches maintain similar ranking as in the native or the annealed starches (Table 1).

3.2 Freeze-thaw stability

Freeze–thaw stability results, calculated as % syneresis (amount of separated water), was determined after 4 and 8 days of storage at -20C are presented in Table 2 and 3. The amylose content of the tested starches ranged from 20.4 to 25.0%, where

Table 2. The effect of germinated sorghum extract (GSE) on the %syneresis of starch after 4 days.

		40°C				
		C. P	C.S	T.B	W.S	S.P.S
30 min	No GSE	0.48 ^{b} ± 0.2	0.20 ^{c}± 0.01	0.69^{a} ± 0.2	0.28 ^{c} ± 0.2	0.05 ^{d} ± 0.01
	0.1 mL	0.42^{b} ± 0.04	0.20 ^{c} ± 0.1	0.31^{b} ± 0.2	0.26 ^{c} ± 0.1	0.02 ^{d} ± 0.01
	1.0 mL	0.07^{c} ± 0.01	0.17 ^{b} ± 0.1	0.19^{b} ± 0.1	0.20 ^{a} ± 0.1	0.04 ^{d} ± 0.01
60 min	No GSE	0.29 ^{c} ± 0.1	2.28 ^{a} ± 0.4	1.14^{b} ± 0.8	0.19 ^{d} ± 0.1	0.15 ^{d} ± 0.03
	0.1 mL	0.20 ^{b} ± 0.2	0.16 ^{c} ±0.2	0.92^{a} ± 0.4	0.17 ^{c} ± 0.1	0.03 ^{d} ± 0.01
	1.0 mL	0.06^{c} ± 0.01	0.05^{c} ± 0.01	0.33^{a} ± 0.2	0.12 ^{b} ± 0.1	0.01 ^{d} ± 0.01
		50°C				
30 min	No GSE	0.71^{c} ± 0.02	0.41^{d} ± 0.03	0.82^{b} ± 2.1	0.94^{a} ± 0.1	0.29 ^{e} ± 0.1
	0.1 mL	0.40 ^{b} ± 0.1	0.12 ^{c} ±0.1	0.70^{a} ± 0.2	0.13^{c} ± 0.1	0.18 ^{c} ± 0.1
	1.0 mL	0.12^{c} ± 0.05	0.07^{d} ± 0.01	0.40^{a} ± 0.1	0.12^{c} ± 0.1	0.28 ^{b} ± 0.01
60 min	No GSE	1.20 ^{b} ± 0.7	0.50^{d} ± 0.02	1.40^{a}±1.1	0.92^{c} ± 1.3	0.19 ^{e} ± 0.01
	0.1 mL	0.48^{c} ± 0.02	0.20^{d} ± 0.02	0.80^{a} ±0.1	0.82^{a} ± 2.1	0.18 ^{e} ± 0.06
	1.0 mL	0.33^{b} ± 0.03	0.05^{d} ± 0.01	0.20^{c}±0.1	0.42 ^{a} ± 1.6	0.03 ^{e} ± 0.02
		60°C				
30 min	No GSE	0.72^{d} ± 0.01	1.95 ^{b} ± 1.2	2.80 ^{a} ± 2.3	Gelatinized	1.00 ^{c} ± 0.6
	0.1 mL	0.09^{d} ± 0.01	0.89 ^{a} ± 0.4	2.22^{a} ± 1.6	Gelatinized	0.23^{c} ± 0.01
	1.0 mL	0.04^{d} ± 0.01	0.59 ^{b} ± 0.1	1.43^{a} ± 0.1	Gelatinized	0.19 ^{c} ± 0.01
60 min	No GSE	0.69 ^{c} ± 0.1	2.21 ^{b} ± 1.6	5.94^{a} ± 1.6	Gelatinized	2.66 ^{b} ± 1.2
	0.1 mL	0.40^{c} ± 0.02	1.07 ^{b} ± 1.1	5.22^{a} ± 2.3	Gelatinized	0.14 ^{d} ± 0.2
	1.0 mL	0.34 ^{c} ± 0.1	0.50 ^{b} ± 0.2	3.97^{a} ± 2.1	Gelatinized	0.18 ^{d} ± 0.06

Data flowed by the same letter across rows are not significantly different. [1]GSE=germinated sorghum extract; [2]consistency index (*K*), [3]flow behavior (*n*) index, [4]C.P = chickpea; [5]C.S=corn starch; [6]T.B=Turkish beans; [7]S.P.S= sweet potato starch. The r^2 range was: for C.P 0.93-0.99, C.S 0.87=0.99, S.P.S 0.92-0.99,and T.B 0.95-0.99. Values followed by different letters within each raw are significantly different ($p \leq 0.05$).

Table 3. The effect of germinated sorghum extract (GSE) on the %syneresis of starch after 8 days.

		40°C				
		C. P	C.S	T.B	W.S	S.P.S
30 min	No GSE	1.31^{c} ± 0.9	0.53^{d} ± 0.2	8.58^{a} ± 2.1	0.39 ^{e} ± 0.02	3.68^{b} ± 1.2
	0.1 mL	0.03^{d} ± 0.01	0.49 ^{b} ± 0.1	3.76^{a} ± 1.4	0.37 ^{c} ± 0.02	0.04^{d} ± 0.01
	1.0 mL	0.67 ^{b} ± 0.3	0.18^{d} ± 0.1	2.63^{a} ± 1.2	0.30 ^{c} ± 0.01	0.35^{c} ± 0.1
60 min	No GSE	1.76 ^{c} ± 0.8	0.58^{d} ± 0.2	7.42 ^{a} ± 2.1	1.97 ^{c} ± 0.80	3.13^{b} ± 2.1
	0.1 mL	0.37 ^{e} ± 0.2	0.53^{d} ± 0.1	4.69^{a} ± 2.3	1.65 ^{b} ± 0.70	0.63^{c} ± 0.3
	1.0 mL	0.33 ^{c} ± 0.1	0.07^{d} ± 0.01	1.70^{a} ± 1.1	1.07 ^{b} ± 0.90	0.37 ^{c} ± 0.1
		50°C				
30 min	No GSE	2.30 ^{c} ± 1.10	2.90 ^{b} ± 1.3	13.6^{a} ± 2.30	0.56^{e} ± 0.03	0.96 ^{d} ± 0.3
	0.1 mL	1.00 ^{b} ± 0.40	0.2^{0d} ± 0.10	3.50^{a} ± 1.20	0.68 ^{c} ± 0.20	0.69 ^{c} ± 0.1
	1.0 mL	0.30^{b} ± 0.01	0.20^{c}±0.01	2.50^{a} ± 1.60	0.26 ^{c} ± 0.01	0.04^{d} ± 0.01
60 min	No GSE	3.01 ^{d} ±0.80	6.10 ^{c} ± 2.1	16.8^{a} ± 3.40	14.9 ^{b} ± 0.21	1.89 ^{e} ± 1.6
	0.1 mL	1.02 ^{d} ± 0.01	1.22 ^{c} ± 1.1	6.80^{a} ± 2.10	3.86 ^{b} ± 1.60	0.22^{e} ± 0.02
	1.0 mL	0.73^{c} ± 0.21	0.36^{d} ± 0.02	4.10^{a} ± 1.30	1.41^{b} ± 1.10	0.04^{e} ± 0.01
		60°C				
30 min	No GSE	2.39 ^{c} ± 1.2	2.73^{c} ± 1.30	5.00 ^{a} ± 2.1	Gelatinized	4.67 ^{b} ± 1.3
	0.1 mL	1.00 ^{c} ± 0.5	0.98^{d} ± 0.03	9.05 ^{a} ± 3.2	Gelatinized	3.20 ^{b} ± 2.1
	1.0 mL	0.37 ^{d} ± 0.1	0.89^{c} ± 0.04	9.19 ^{a} ± 3.1	Gelatinized	2.17 ^{b} ± 1.2
60 min	No GSE	3.1^{c} ± 1.30	2.34^{d} ± 1.60	9.19 ^{a} ±2.1	Gelatinized	5.62 ^{b} ± 2.1
	0.1 mL	1.21 ^{b} ± 1.4	1.96 ^{c} ± 1.21	10.31^{a} ±2.6	Gelatinized	1.59 ^{cd} ± 0.9
	1.0 mL	1.05 ^{d} ± 1.1	1.61 ^{b} ± 1.10	5.64 ^{a} ± 2.5	Gelatinized	1.16 ^{c} ± 0.7

Data flowed by the same letter across rows are not significantly different. [1]GSE=germinated sorghum extract; [2]consistency index (*K*), [3]flow behavior (*n*) index, [4]C.P = chickpea; [5]C.S=corn starch; [6]T.B=Turkish beans; [7]S.P.S= sweet potato starch. The r^2 range was: for C.P 0.93-0.99, C.S 0.87=0.99, S.P.S 0.92-0.99, and T.B 0.95-0.99. Values followed by different letters within each raw are significantly different ($p \leq 0.05$).

C.S, T.B, S.P.S, C.P, and W.S, exhibited 20.4±0.07%, 20.9±0.08, 22.6±0.04, 24.0±0.05, and 25.0±0.06, respectively. The syneresis of annealed starches without GSE varied according to their botanical origin, the number of freeze-thaw cycles, annealing temperature and time.

In a previous publication (Alamri et al., 2013), the authors reported %syneresis before annealing ranged from 0.12 to 19.1% along these values: C.S=17.1, T.B=19.1, S.P.S=0.12, C.P=15.5, and W.S=7.5, but the data presented here is much lower for the same starches, where the range of % syneresis was 0.05-2.28%, 0.19-1.20%, and 0.69-5.94% for samples annealed at 40, 50, and 60˚C, respectively. The underline cause for the low syneresis could be attributed to starch annealing prior cooking. Starch samples annealed at 40˚C for 30 min and stored for 4 days, exhibited different syneresis and ranked as: C.P>T.B>W.S>C.S>S.P.S, but at 60 min, at the same temperature, the ranking was: C.S>T.B>C.P>W.S>S.P.S (Table 2). This ranking showed that annealing time at the same temperature is a major factor that affects starch gel syneresis and can neutralize the role of high amylose content on syneresis. This was factual, because the amylose content of C.P was relatively high (24.0), but exhibited the highest syneresis after 30 min annealing, whereas after 60 min it ranked after T.B (22.9). Therefore, annealing physically modified starch granule structure in a way that reduced amylose retrogradation (Alqah et al., 2020). At 50˚C annealing, high amylose starches (W.S and C.P) had the highest syneresis. Once again, C.P starch appeared to be extra sensitive to annealing time. After annealing, starches with the lowest amylose content (T.B and C.S) exhibited the highest syneresis compared to those with high amylose content. In fact, starch gel hardness is physically initiated by amylose retrogradation and the gel becomes enzyme and heat resistant (Jane, 2009). Other literature reports showed that amylose content was the determining factor for syneresis, where higher amylose content is associated with higher syneresis (Srichuwong et al., 2005a). The same authors showed that, amylose content was correlated with starch gelatinization, pasting properties, swelling power, retrogradation, gel hardness, and in vitro digestibility (Srichuwong et al., 2005b). Thereby, the data presented here showed that annealing is a formative factor on gel syneresis not amylose content, but it is possible that amylose chains length and amylopectin degree of branching can be additional factors together with annealing in limiting the occurrence of syneresis. Previous work also showed how amylose short chains are correlated with decrease in gelatinization temperature and swelling power, and increase in enzyme digestibility of the granule (Srichuwong et al., 2005a), since during long term storage of cooked starches, the short chains DP 6–12 have been reported to delay retrogradation. In general, starch gel syneresis increases with increase in freeze-thaw cycles, and varied along botanical origin. Therefore, intense amylose retrogradation can accelerates phase separation, thus increases gel syneresis. After 8 days of storage at -20˚C, T.B and SPS had the highest syneresis and W.S was the least, whereas C.P was in between. Unlike C.S, T.B syneresis appeared to be independent of temperature or annealing time. After GSE was added, all samples exhibited reduction in syneresis regardless of annealing time and temperature, or storage time (Table 3). The reduction was significant for starches annealed at the same temperature and time, but it was not significant for others. As we concluded in earlier work (Alqah et al., 2020), W.S was the most

susceptible to α-amylase attack and to annealing at 60°C among the tested starches, because it gelatinized at this temperature without the addition of GSE. In general, W.S syneresis dropped significantly only when 1.0 mL of GSE was added. The T.B gels had the most syneresis with or without GSE, but the most drop in syneresis was due to GSE rather than annealing (Table 3).

Once again, samples with the highest amylose content did not exhibit the highest syneresis with the addition of GSE. Possibly, the reason for the drop in syneresis after GSE treatment is the increase in the short amylose chains due to α-amylase degradation, which is known to slow down amylose retrogradation, the main cause of syneresis. Therefore, the differences in syneresis between the tested starches can be attributed to the granule structural differences such as the length of amylose chain, amylopectin chain length and proportion of short chains.

3.3 Dynamic oscillation

Previously, researchers recommended the use of G` as a guide to define rheological experimental conditions since it has more processing value than G``, especially for starchy products (Hsu et al., 2000). Figure 1 shows changes in storage modulus (G`) as a function of oscillation frequency of the tested

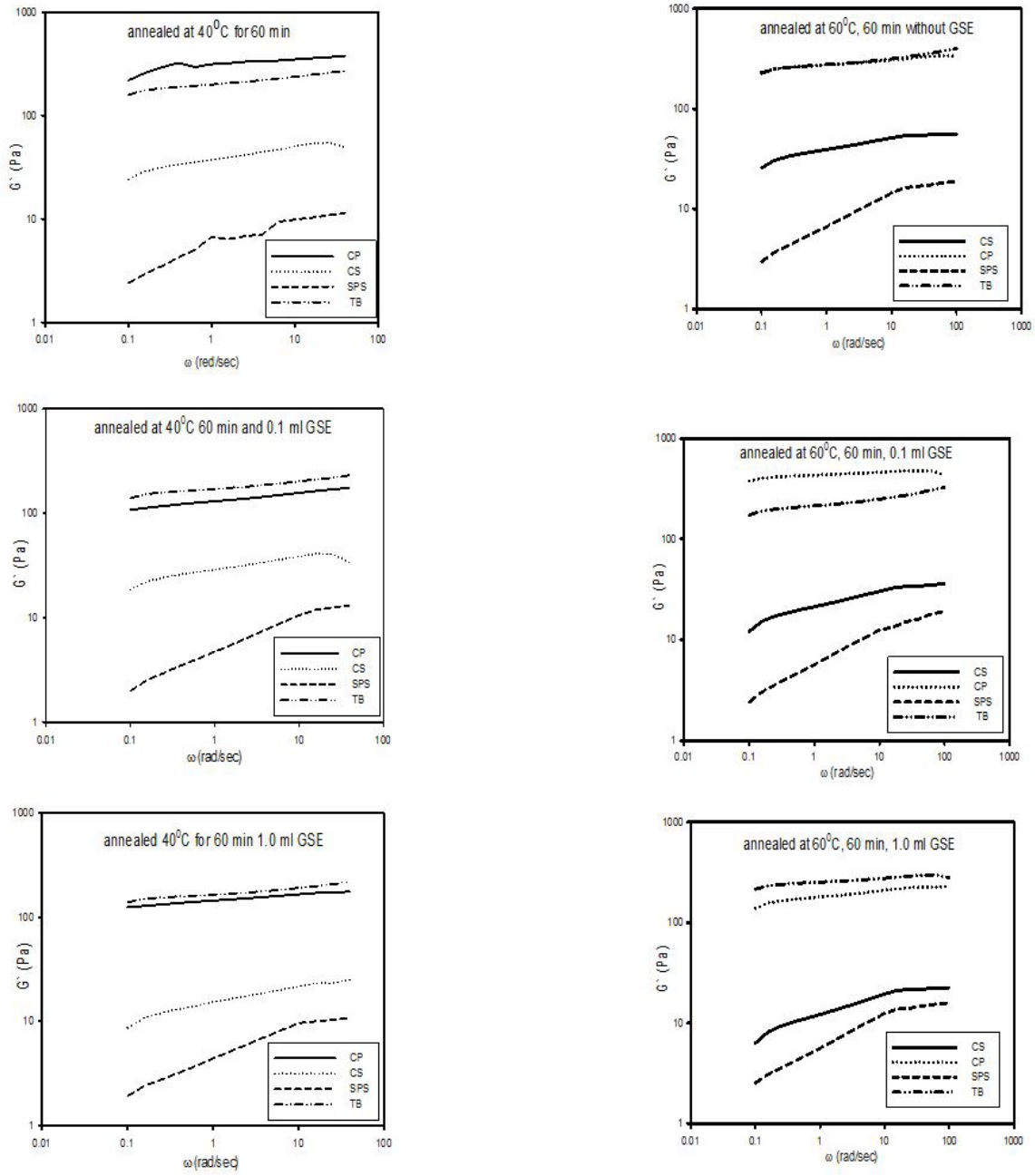

Figure 1. Change in storage modulus (G`) of corn, chickpea, sweet potato, and Turkish beans starches annealed at, 40 °C or 60 °C and 0, 0.1 and 1.0 ml germinated sorghum extract (GSE).

starches annealed at 40 ^{0}C or 60 ^{0}C for 60 min. The storage modulus G` is a measure of the energy stored in the material per deformation cycle. The measure of changes to these parameters can be ascertained by instituting optimum testing conditions (oscillation frequency, strain, and temperature) and establishing the linear viscoelastic region (LVE). In this work, the LVR was determined within wide range of the experimental conditions, however, 5% strain between 25^0 C and 70 °C was found to be within the LVR. Other researchers used up to 50% strain at a range of 10 to 47 (rad/sec) frequencies for their precooked starch water systems (Lagarrigue & Alvarez, 2001). Therefore, 5% strain used in this study, is low enough to be within the LVR and allow for defining the gel properties without damaging its structure. Regardless of annealing temperature, time and GSE level, starch gels are divided into two groups with respect to G` (Figure 1). One group with higher G` (T.B and C.P) and another with lower G` (C.S and S.P.S). The difference in G` between the highest (T.B) and the lowest (S.P.S) was 89%. At higher frequencies, the G` of T.B exceeded C.P by 23%, C.S by 92%, and S.P.S by 95%, whereas at lower frequencies the gap was even larger (Figure 1). The data presented in Table 3 showed G` increase of T.B and S.P.S due to annealing, but a reduction in G` was recorded for C.P and C.S. The increase in G` indicates that annealing of native starches without GSE increased the elastic behavior of the starches by changing the basic structurer of the granule during annealing. After annealing at 40 ^{0}C without GSE, the increase in G` of T.B and S.P.S indicates a more oscillation dependent gel compared to annealing at 60 $^0$C, where G` decreased (Table 4). All samples annealed at 60 ^{0}C in GSE exhibited increase in G` compared to the control demonstrating more oscillation dependent compared to 40 ^{0}C. This shows the direct effect of α–amylase on the granule structure which is reflected on the network structure and elasticity of the gel. At lower oscillation, C.S and S.P.S were more oscillation dependent than at higher frequencies, but the gap between the G` is narrower at higher frequencies. Annealing temperature had a direct effect on the magnitude of G` change, because the slope of increase in G` as a function of oscillation frequency for C.S and C.P was smaller at 40 ^{0}C compared to other temperatures.

The slope of G` was also smaller for C.S and T.B at 60 $^0$C compared to other temperatures (Figure 1). On the other hand, the highest slope was recorded for S.P.S signifying the most sensitivity to oscillation among the tested starches. In addition, the S.P.S profile showed crossover between G` and G`` for all experimental conditions (profiles are not shown). The effect of annealing on G` was different according to starch type, because longer annealing time appeared to increase the G` of all starches except for T.B (Table 4). The variation of the effect of annealing on G` can be attributed to the different granule structure, molecular size of amylose/amylopectin and granule porosity. The effect of longer annealing time in the presence of GSE varied across starch type, as well, since all starches exhibited reduction in G` at higher GSE (Table 4). The highest G` was recorded for T.B and C.P and the lowest was for S.P.S. The most sensitive starch to α–amylase with respect to change in G` was C.P, because it exhibited the most reduction in G` as a function of enzyme level (extraction volume added) indicating softer gels. Therefore, annealing effect on the granule structure is different for each starch, where G` reduction indicates gels with reduced solid like behavior at higher GSE. This could be

Table 4. The effect of germinated sorghum extract (GSE) on the Storage modulus (G`) of starches at 6.30 (rad/sec) annealed at 40, 50, and 60°C

		C. P	C.S	T.B	S.P.S
		40 ^{0}C			
	Native	202.7 ± 30.8	45.5 ± 0.11	237.2 ± 25.0	3.9 ± 0.03
30 min	No GSE	183.7 ± 17.2	31.1 ± 0.09	243.3 ± 33.6	9.4 ± 0.01
	0.1 mL	158.1 ± 21.5	28.5 ± 0.60	230.1 ± 27.4	8.9 ± 0.01
	1.0 mL	102.4 ± 8.00	26.9 ± 0.08	190.1 ± 21.8	8.1 ± 0.02
60 min	No GSE	342.4 ± 27.2	47.2 ±0.12	229.4 ± 19.0	9.4 ±0.01
	0.1 mL	160.6 ±11.0	36.3 ± 0.31	193.3 ±19.8	8.9 ± 0.01
	1.0 mL	149.8 ±19.0	20.0 ± 0.1.67	182.9 ± 15.7	8.2 ± 0.03
		50 ^{0}C			
30 min	No GSE	197.1 ±12.0	36.4 ± 1.10	263.6 ± 31.0	9.4 ± 0.01
	0.1 mL	175.3 ± 22.0	32.6 ±0.09	260.3 ± 26.9	8.2 ± 0.01
	1.0 mL	137.6 ± 13.0	20.8 ± 0.81	222.6 ± 34.6	6.6 ± 0.04
60 min	No GSE	211.6 ± 28.1	36.4 ± 1.88	268.7 ± 45.1	10.6 ± 0.02
	0.1 mL	171.5 ± 11.2	31.2 ± 1.96	265.1 ± 40.7	11.8 ± 0.01
	1.0 mL	161.8 ± 23.5	21.1 ± 0.87	219.5 ± 38.9	9.6 ± 0.01
		60 ^{0}C			
30 min	No GSE	458.2 ± 34.9	37.4 ± 0.79	383.8 ± 29.5	10.7 ± 0.01
	0.1 mL	382.6 ± 42.8	25.0 ± 2.01	303.7 ± 22.8	9.0 ± 0.03
	1.0 mL	296.5 ± 33.7	25.6 ± 0.45	249.8 ± 35.9	9.5 ± 0.03
60 min	No GSE	451.2 ± 51.3	48.5 ± 0.77	304.9 ± 19.7	12.4 ± 0.01
	0.1 mL	298.3 ± 37.1	28.2 ± 1.21	271.1 ± 24.0	10.7 ± 0.04
	1.0 mL	203.2 ± 44.2	17.7 ± 0.07	239.1 ± 17.8	10.7 ± 0.04

C.P = chickpea; C.S =corn starch; T.B ='Turkish beans; S.P.S =sweet potato starch.

accredited to reduction in the length of amylose chains due to the action of α–amylase. Shorter amylose chains limit the formation of strong network and weaken the structure of the gel by reducing the number of available hydrogen bonding, thus the produced gel is softer. Generally, starches are different with respect to their structure on the surface of the granule which is reflected on the porosity and the mechanism of α–amylase attack. Thereby, tightly packed surface forces exo-hydrolysis, whereas porous surface allows for enzyme penetration. This leads to internal hydrolysis which causes significant change to the final gel formed after gelatinization. It is well established that, unlike B-Type starch, the granules of A-type starches are more porous which allow for enzyme penetration. Consequently, preferential hydrolysis of the amorphous region is common for A-type, which is common for cereal starches (mostly A-type) and not possible for potato starch (Blazek and Gilbert, 2010). The same authors reported increase in DSC peak temperature of enzymatically hydrolyzed starches. The data presented here showed limited effect of GSE on the G` of S.P.S (tuber) compared to the other starches (Table 4).

The tan δ, which is the ratio of G``/G`, is a characteristic parameter used for evaluation of the viscoelastic behavior of gels. Predominantly elastic behavior is indicated by tan δ <1, while a

tan δ >1 indicates predominantly viscous behavior. The addition of GSE had direct effect on tan δ where the elasticity of the gels was reduced especially for C.S and C.P. The tan δ of these starches was >1 (data is not shown), but some of the tested gels were more elastic than others based on tan δ values. The tan δ rang was, C.S (0.16-0.47), C.P (0.06-0.16), S.P.S (0.039-0.90), and T.B (0.05-0.21). Therefore, S.P.S was the least elastic gel, since it has the least G` and the highest tan δ, as mentioned earlier, and C.P was the most elastic.

The log of complex viscosity (η*) versus oscillation frequency plot shows shear-thinning behavior. Such behavior is in good agreement with those found for other starches regardless of botanical origin, but with different magnitude (Yoo et al., 2005). All starches exhibited reduction in η* as a function of oscillation frequency. Figures 2 and 3 show η* profiles of C.P and T.B as examples, since the remaining starches exhibited similar profiles, but with different

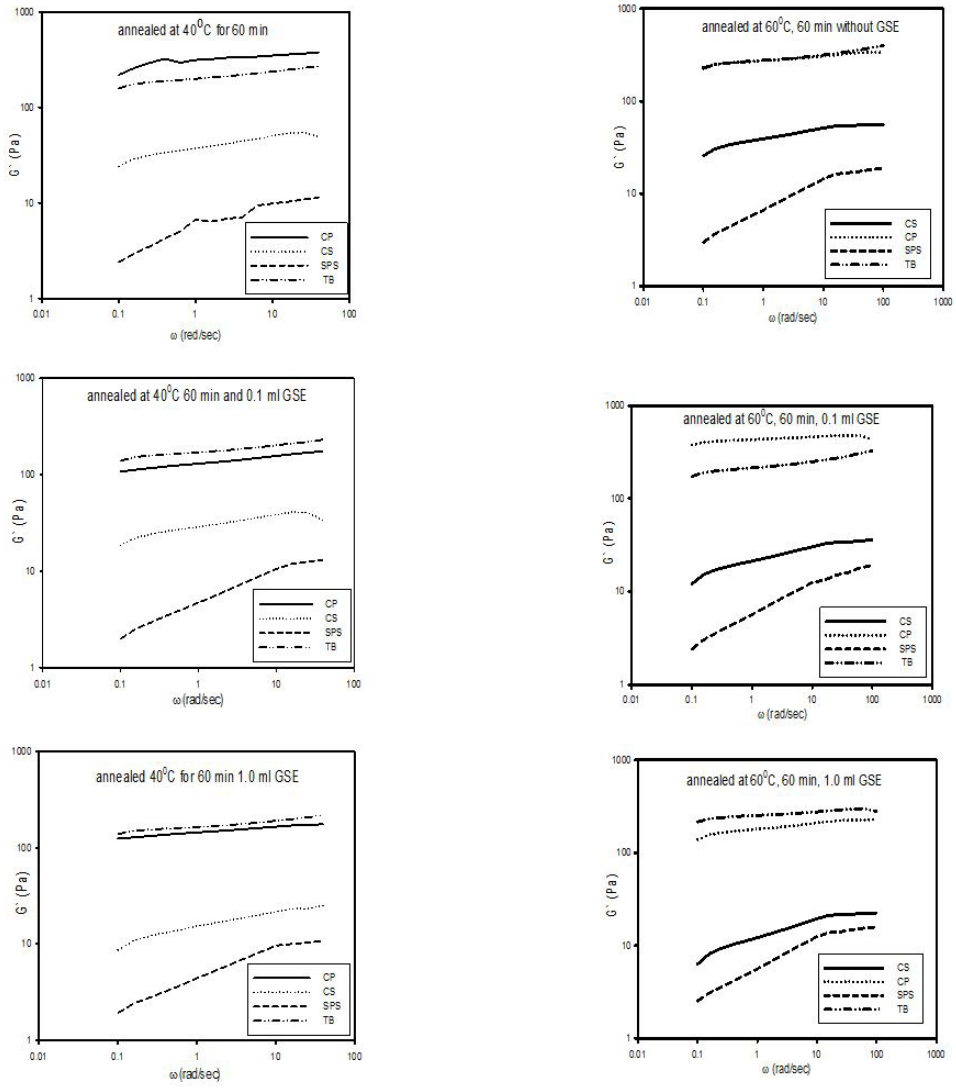

Figure 2. Complex viscosity of chickpea starch annealed at 40, 50, and 60 °C for 30 and 60 min and treated with germinated sorghum extract (GSE).

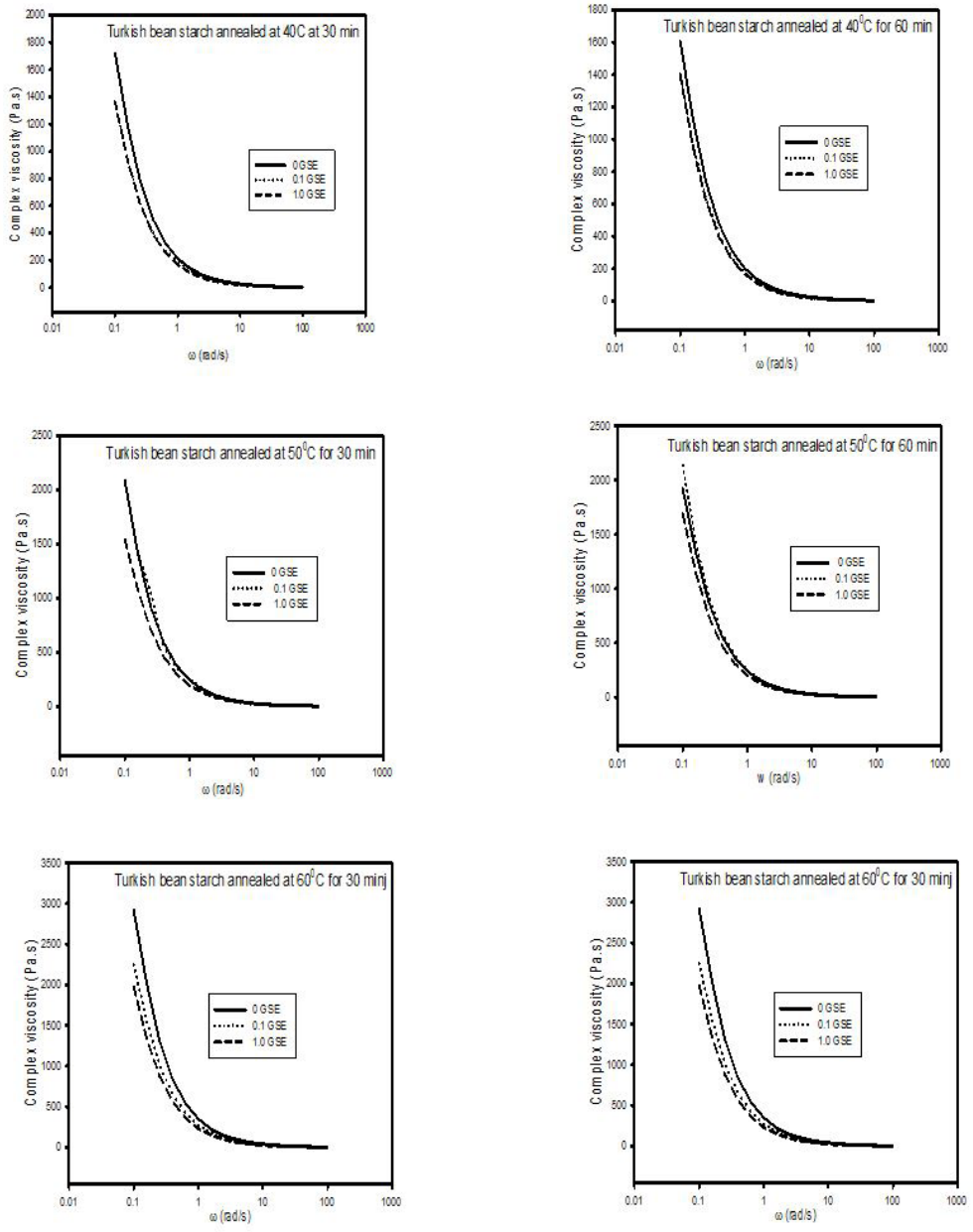

Figure 3. Complex viscosity of Turkish bean starch annealed at 40, 50, and 60 ^{0}C for 30 and 60 min and treated with germinated sorghum extract (GSE).

magnitude. The addition of GSE reduced the η* which is in line with the reduction in G` at higher GSE, therefore, the starch gel became less elastic. The profiles in Figure 2 show clear reduction in η* of C.P compared to T.B, but higher annealing temperature and longer annealing time seemed to close the gap between the samples treated with different GSE. The gap between η* profiles of T.B (Figure 3) are closer to each other indicating limited change in η* at higher GSE compered to C.P starch profiles (Figure 2).

4 Conclusion

Significant differences among the starches from different botanical origin (tuber, legume and cereal) regarding the physicochemical and rheological properties as a result of annealed with or without germinated sorghum extract (GSE). All the starch gels exhibited shear thinning behavior with distinctive more elastic than viscous behavior (G`> G``), except for S.P.S, where a crossover between G` and G`` occurred. Starches with the higher G` included chickpea and Turkish bean, whereas corn and sweet potato starches exhibited lower G` and characterized as less elastic. Due to the sharp increase in G` at low frequencies, corn and sweet potato starches are considered oscillation dependent. The G` of all tested starches was reduced after annealing and further decreased due to GSE. The data showed lower freeze thaw stability and higher water holding capacity after annealing and GSE treatment combined with starch botanical origin. Generally, S.P.S exhibited the lowest freeze thaw stability.

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