Functional properties of defatted chickpea flour heat-induced gels

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Abstract: Defatted chickpea flour (DCF), which is a by-product of chickpea oil extraction industry, is rich in nutrients that are beneficial to human health. In this study, the effects of temperature and DCF variation on the rheological properties, water holding capacity, freeze-thaw stability and microstructure of DCF heat induced gels were investigated. The results showed that the viscoelasticity, frequency dependence, and resistance strength of heat induced gels increased significantly with the increase of temperature and DCF variation. The degree of denaturation and water retention of heat induced gels increased significantly with increased variables within the temperature and variation windows of 75°C to 95°C and 13% to 21%. The CLSM results revealed that variations of both temperature and DCF variation could cause the proteins in the heat induced gels to aggregate gradually and to form protein aggregations. When temperature or variation exceeded certain value (85°C or 17%), the protein aggregations broke up and the protein clusters became smaller and more homogeneous. Therefore, the heat induced gels presented better water holding capacity, viscoelasticity, structural stability and gel property at a temperature of 95°C or a DCF variation of 21% within the present experimental range.

Keywords: defatted chickpea flour, rheological property, freeze-thaw stability, water holding capacity, microstructure

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1 Introduction

Chickpea (*Cicer arietinum Linn*.), rich in protein and dietary fiber, is often used in the field of food ingredient and additive, due to its unique flavor and excellent nutritional characteristics[1-3]. Chickpea is proved to be highly beneficial to human health since its specific components are medically effective for the treatment of diseases such as hypertension, hyperlipidemia, and hypercholesterolemia[4-6]. Defatted chickpea flour (DCF) is a byproduct of the chickpea oil extraction industry and proved to show many excellent functional properties[7,8]. The heat-induced gel formation process and the final gel properties are mainly influenced by the heat treatment temperature program and the concentration of active ingredients. Adjusting the heat treatment temperature program and solute concentration can lead to different degrees of modification of proteins, starch, dietary fibre, etc[9,10].

The related research of chickpea is gradually increasing and deepening because of its advantages of high nutrition and easy absorption. Wang et al.[4] discussed the active substances and molecular mechanism in chickpea and found that chickpeas have many functional components that other legumes do not have. Which can affect the application property of chickpea. Then Mesfin et al.[11] studied the effect of sprouting, roasting and variety on the performance of the chickpea protein isolate powder, and finally found that the chickpea protein isolate can be used as a beneficial ingredient for functional food in the food industry. The pasting-melting transformation of chickpea starch was investigated by Lefèvre et al.[12], which found that chickpea starch could successfully undergo a melt transformation regardless of its moisture content. He et al.[13] discussed the isolated aquafaba from chickpea and found that it contains various nutritional factors such as proteins, polysaccharides and minerals, which can be applied in food products to improve foaming and emulsification.

Many studies have attempted to investigate the effects of temperature and concentration in legume plant-based materiel foods on the composition of protein, starch, etc. and the modification of the components, which is more in line with product demand. Jiang et al.[14] studied the emulsification property of heat-induced aggregation of soy protein isolate (SPI) and found that preheating was significantly associated with the stability and emulsification property of the heat-induced SPI aggregation. In another example, Ji et al.[15] studied the fibrosis of SPI in terms of viscosity, emulsifying property and structure, which was proportional to temperature. Beliciu and Moraru[16] investigated the effect of heating on micellar casein-soy protein mixture and found interesting rheological properties of the mixture after heat treatment. Variations in the concentration of the mixture around the critical concentration resulted in large changes in the rheological properties. Lin et al.[17] investigated the gel properties of SPI, alginate and oil, and the final results showed that the concentrations of these components were significantly correlated with gelation.

To our knowledge, there are few studies related to the effects of heating temperature and DCF concentration on heat-induced gels of defatted chickpeas. Therefore, the heat treatment temperature (monotonous heating and holding program) and the concentration of DCF were taken as the main research variables to explore the possibility of application of the DCF heat-induced gel in the food industry. The heat-induced gel properties of DCF were analyzed by rheological experiment, freeze-thaw stability test, water-holding test and microstructure, which will provide useful information for the applications of DCF in the food industry.

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2 Materials and methods

2.1 Materials

DCF was purchased from Shaanxi Panier Biotechnology Co., Ltd. (Shaanxi, China); Rhodamine B (BS) was purchased from Shanghai McLean Biochemical Co., Ltd. (Shanghai, China).

2.2 Sample preparation

2.2.1 Preparation of heat-induced gels of defatted chickpea with different heating temperatures and DCF concentrations

At room temperature of 25°C, a certain amount of deionized water and DCF, were weighed into the bealcer using an electronic balance, and stirred on a magnetic stirrer at 800 r/min for 15 min, which are fully dissolved to prepare chickpea flour solutions with concentrations of 13%, 15%, 17%, 19%, and 21%, of which six parts of the 17% DCF solution are sealed with plastic wrap.

The five 17% DCF solutions were heated in a water bath with different temperature (75°C, 80°C, 85°C, 90°C, and 95°C for 20 min). After heating, it was taken out and cooled to room temperature to prepare chickpea heat-induced gels with different heating temperatures. Each solutions with different DCF concentrations (13%, 15%, 17%, 19% and 21%) were heated in a water bath at 95°C for 20 min. After heating, it was taken out and cooled it to room temperature to prepare chickpea heat-induced gels with different concentrations.

2.2.2 Preparation of CLSM samples from heat-induced gelation of DCF at different temperatures and concentrations

The 15 mL of 17% DCF solution added 10 μL of 2 mg/mL Rhodamine B dye was stirred using a magnetic stirrer to prepare five identical solutions, which were then heated in a water bath at different temperatures (75°C, 80°C, 85°C, 90°C, and 95°C) for 20 min. When the heating has completed, it was take out and cooled to room temperature. The same amount of stain was added to 15 ml of five different concentrations of DCF (solutions 13%, 15%, 17%, 19%, 21%) and stirred well. Placed in a water bath at 95°C for 20 min, and then cooled to room temperature to prepare different DCF heat-induced gel CLSM samples.

2.3 Experimental methods

2.3.1 The rheology tests

This sample was rheologically tested using an AR2000ex rotational rheometer. An aluminum plate fixture with a diameter of 40 mm was selected to track the heat-induced gel process, and measure its functional property. The distance between the fixture and the Peltier plate was kept at 1mm during the test. A Peltier device with a water circulation system was used for the experiment. The temperature during the process is controlled. During the experiment, a little silicone oil was dropped on the edge of the sample on the Peltier plate to prevent the sample from evaporating.

1) Strain sweep test

The strain sweep experiment was performed at a fixed frequency of 1 Hz at room temperature, and the observed strain ranged from 0.1% to 100%. The viscoelasticity of all samples was observed through the data, which were recorded every loss, and the linear viscoelastic region (LVR) was determined[18].

2) Frequency sweep test

The frequency sweep experiment was performed at room temperature and the observed angular frequency range was 1-1000 rad/s. To ensure that the strain amplitude of the measured sample was stable in the linear viscoelastic region, the strain value was kept at 1%. The frequency dependence of storage modulus G′ and loss modulus G″ is approximated by Equations (1) and (2):

$$G' = K' \cdot w^{n'} \quad (1)$$

$$G'' = K'' \cdot w^{n''} \quad (2)$$

where, K' and K'' represent the power-law exponent; w represents the angular frequency; n' and n'' are the frequency exponent, which represents the frequency dependence of the storage modulus G' and the loss modulus G''[19].

3) Creep recovery test

The creep recovery experiment was carried out at room temperature with a stress of 7.958 Pa applied to the heat-induced gel sample. The first 120 s was the creep stage. After this stage, the stress applied to the sample was removed and the recovery stage was entered, which was also 120 s, deformation data and strain recovery data throughout the experiment were recorded[20].

2.3.2 Freeze-thaw stability test (DSC)

Remove samples that have been in the freezer overnight. The sample (6-12 mg) was weighed by an electronic balance, and placed into an aluminum pan and placed outside the DSC oven cavity. An empty aluminum pan was set as a control group and placed on the other side of the furnace cavity. The nitrogen flow rate was set to 200 mL/min. The temperature was lowered from 40°C to –40°C at a cooling rate of 10°C/min, and then raised to 40°C at the same rate. Heat changes were recorded as low with temperature during heating and cooling. Three parallel experiments were ran per sample[21].

2.3.3 Water retention test

The water holding capacity test was performed by centrifugation in a centrifuge at room temperature. A 50 g of sample was placed in a centrifuge tube and placed in a centrifuge with a rotating speed of 5000 r/min for 15 min[22].

2.3.4 Confocal microscopy

The microstructure of heat-induced gel was doserved by confocal microscopy. An appropriate amount of the stained sample was placed on a slide and covered with a coverslip. The sample was observed upside down on a 60x objective lens coated with silicone oil. The excitation wavelength of rhodamine B was set at 552 nm and the emission wavelength was between 570 and 675 mm during the observation[23].

3 Results

3.1 Strain sweep test results

Figure 1a represents photographs of heat induced gels at different DCF concentrations (From left to right, the DCF concentrations were 13%, 15%, 17%, 19%, and 21%). It can be seen that the gels are solidified more obvious with increasing DCF concentration. Figures 1b and 1c show the effect of heating temperature on the strain scan of heat induced gels and the variation of the G' and G''. The length of the linear viscoelastic region (LVR) of the heat induced gel at a heating temperature of 75°C is slightly shorter than at the other heating temperatures (80°C, 85°C, 90°C, 95°C). This may be because the higher heating temperature enhanced the structure of the gel system and increased the resistance strength of the gel system[18]. Therefore, the gel heated at 75°C reached a certain value (the point of stractured collapse) was faster.

The G' and G'' of the heat induced gel showed in a steady state at applied strain below 1%. The G' gradually decreased from 1 to 100% strain, while the G'' showed an increasing tendency to a maximum and then decreased in this region. The peak at 75°C was lower than the peaks at other heating temperatures, which may be caused by the higher temperature disrupting the complex equilibrium of the internal structure of the heat induced gel[19].

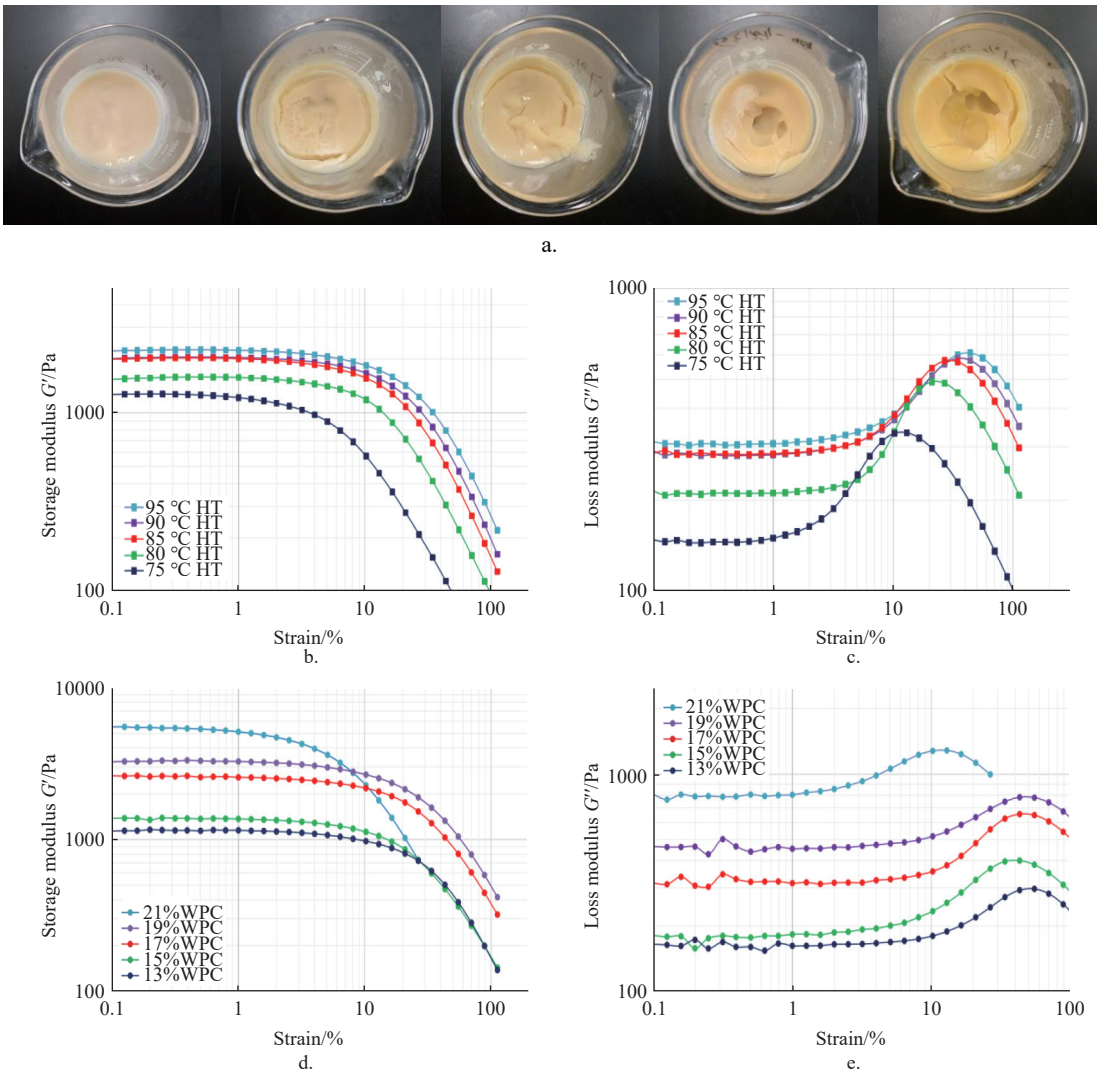


Figure 1 Effects of heating temperature (a and b) and concentration (c and d) on DCF heat-induced gel strain sweep, storage modulus G' and loss modulus G'' as a function of strain; DCF gels with different concentrations photo (e)

Figure 1d depicts the effect of DCF concentration on the strain scan of the heat induced gel, and Figure 1e shows the variation of the energy G' and G'' with strain. The linear viscoelastic region (LVR) lengths of the heat induced gels at 21% DCF are slightly shorter than other concentrations, which is similar to the effect of different temperatures on the heat induced gels of DCF. This indicated that temperature and concentration have the same trend of influence on the heat induced gels of defatted chickpea. Figure 1d and E also present that as the concentration of DCF increases, the values of the G' and G'' of the heat induced gels gradually increase, which means the elasticity and viscosity of the heat induced gel also increase. This may be due to the fact that the higher the concentration of DCF was the more solute the solution contained, which allows more solute to bind with water to form a more ordered and more compact gel network structure. This resulted in a significant increase in the viscoelasticity and strength of the DCF heat induced gel, which is similar to the phenomenon reported by Huang et al.[24]

3.2 Frequency sweep test results

Figure 2a shows the effect of heating temperature on the sweep frequency of the heat induced gel, and Figure 2b presents the variation of G' and G'' with the angular frequency. It can be seen that G' and G'' of the heat induced gel gradually increase as the heating temperature increases, and the growth rate of G'' is much faster than that of G''. This illustrated that the higher the heating temperature, the greater the denaturation of the heat induced gel and the more viscous it is. This may be because the heating temperature is proportional to the ability of the heat induced gel to weaken non-covalent bonds and to reduce intermolecular interactions. A similar phenomenon was observed by Hu et al[25], who they studied the effect of ultrasound on soybean isolate protein dispersions.

Figure 2c depicts the effect of concentration on the sweep frequency of the defatted chickpea heat induced gel and Figure 2d shows the variation of G' and G'' with angular frequency. G' and G'' of heat induced gels are proportional to the concentration. G' and G'' of the heat induced gels with high DCF concentration are significantly higher than those with low DCF concentration, which indicated that the network structure and mechanical property of the heat induced gels with higher DCF concentrations were stronger and the viscoelastic modulus was greater. This was probably due to the fact that the distance between the particles was reduced by the higher DCF concentration. As a result, the interaction between the particles increased and the viscoelasticity of the gel was improved, which was similar to the conclusion by Bi et al.[26]

As can be seen in Table 1, the values of K' and K'' for heat-induced gels increased significantly with the increase of the heating temperature of heat-induced gels ($p<0.05$). This may be because the increased temperature enhanced the viscosity and elasticity of the

heat-induced gel, which was similar to the conclusion of Tang et al.[19] It was shown that higher heating temperature enhanced the stability of the network structure of heat-induced gel. In Table 1, the higher the concentration of DCF for the heat induced gel, the larger the values of K' and K''. This observation suggested that the increased concentration of DCF contributed to the formation of a more homogeneous and stable gel network structure, which was the same as the results of the previous frequency sweep and strain sweep, in which the viscoelasticity significantly increased with increasing DCF concentration ($p<0.05$). The frequency indices n' and n'' in Table 1 showed relatively small fluctuations, and n'' was always greater than n'. This indicated that both heating and increasing the concentration of DCF were beneficial in enhancing the adhesion of the DCF heat-induced gels. The regression coefficients R^2 for all samples were greater than 95%, which indicated that all samples were well fitted and the selected samples were valid[27].

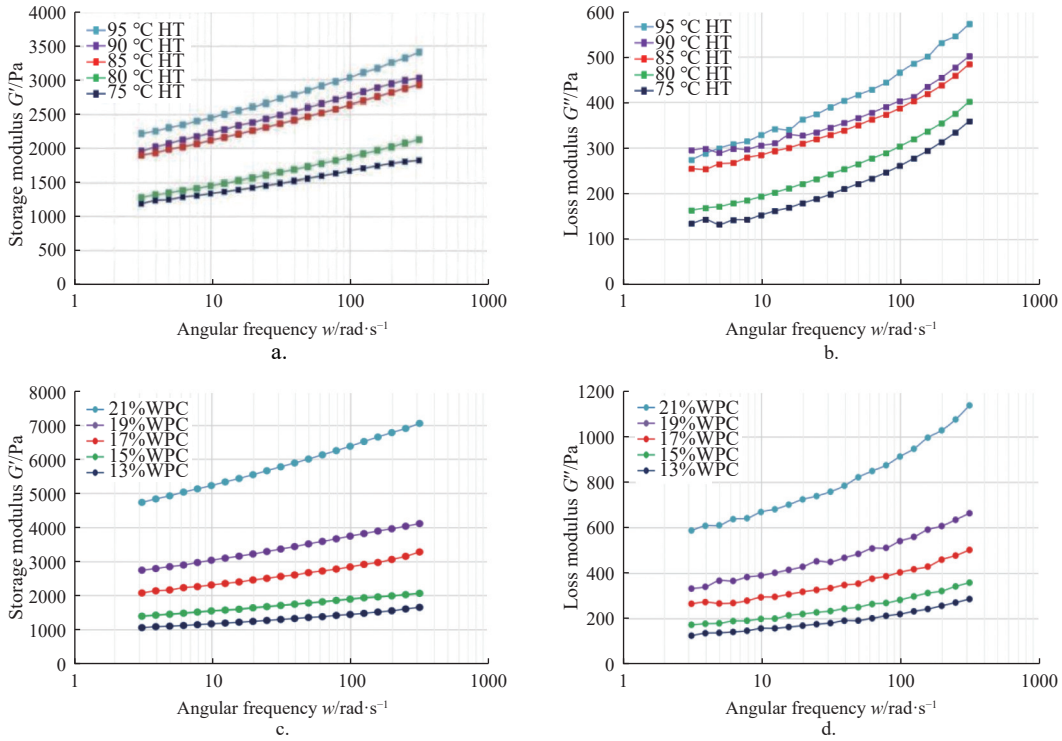


Figure 2 Effects of heating temperature and DCF concentration on the frequency sweep of thermos gel, storage modulus G' and loss modulus G'' as a function of angular frequency

Table 1 Effects of heating temperature and DCF concentration on the formation of heat-induced gels, frequency sweep power law constants K', K'' frequency exponents n', n''

Temperature/°C	$G'=K'\cdot\omega^{n'}$			$G''=K''\cdot\omega^{n''}$		
	K'	n'	R^2	K''	n''	R^2
75	1085.577±3.779^e	0.092±0.001^c	99.80%	90.512±2.217^d	0.234±0.005^a	99.10%
80	1133.081±1.840^d	0.109±0.000^a	100.0%	121.994±1.707^c	0.202±0.003^b	99.60%
85	1703.730±1.502^c	0.095±0.000^b	100.0%	204.240±3.104^b	0.143±0.004^d	98.80%
90	1793.867±8,049^b	0.094±0.001^b	99.70%	232.131±5.774^a	0.124±0.006^e	95.80%
95	1976.756±3.976^a	0.094±0.000^b	99.90%	226.760±2.185^a	0.158±0.002^c	99.60%
Concentration/%						
13	937.463±5.005^e	0.096±0.001^a	99.60%	101.159±1.828^e	0.174±0.004^a	99.00%
15	1267.479±3.641^d	0.086±0.001^c	99.90%	135.944±2.142^d	0.163±0.004^b	99.10%
17	1851.623±11.849^c	0.095±0.002^a	99.50%	206.673±4.097^c	0.148±0.005^c	98.20%
19	2469.747±4.768^b	0.090±0.000^b	99.90%	276.520±3.391^b	0.148±0.003^c	99.30%
21	4287.811±2.654^a	0.086±0.000^c	100.0%	473.537±7.874^a	0.146±0.004^c	98.70%

Note: Values in a column with different superscripts mean significantly different ($p<0.05$).

3.3 Creep recovery test results

Figure 3a presents the effect of different temperatures on the recovery of heat induced gel creep. The strain rate gradually decreases from 0 to 50 s at the beginning. At approximately 50s, the strain rate gradually stabilizes and the strain curve increases linearly. During the recovery phase, the strain gradually stabilizes after a rapid decrease at the moment of stress release. This phenomenon indicated that the strain is highly dependent on the stress[28]. The higher the heating temperature of the heat-induced gel, and the smaller the total strain value of the heat-induced gel, which illustrated that the heating temperature was positively correlated with the heat induced gel elasticity, deformation resistance, and network structure. The performance improved significantly with increasing temperature. In Figure 3a, the higher the temperature the lower the strain value of the thermally induced gel. This revealed that the increasing temperature enhanced the creep properties of the

heat induced gel, which was consistent with the findings of the creep properties of grass carp muscle myofibrillar proteins studied by Huang et al.[29]

Figure 3b shows the effect of different concentrations of DCF on heat-induced gel creep recovery. The overall heat-induced gel deformation decreased with increasing DCF concentration, which indicated that the heat-induced gel presented a stronger network structure at higher solid concentration. This may be due to an increase in solute, leading to increased cohesion in the thermal induced gel network structure, which was in accordance with the phenomenon in the study of the properties of peanut protein isolates by Bi et al.[30]

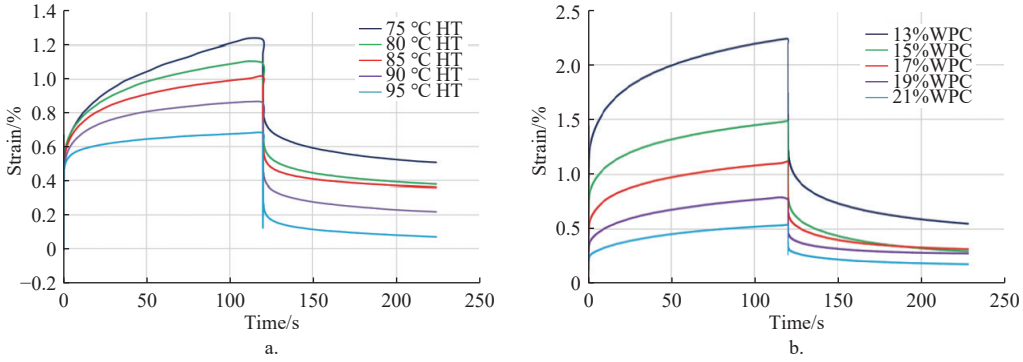


Figure 3 Effects of heating temperature and DCF concentration on heat-induced gel creep recovery

3.4 Freeze-thaw stability test results

Figure 4 represents the freeze-thaw stability of DCF heat-induced gel as a function of temperature. The process is divided into two phases, heating and cooling. The cooling phase is the cooling down from 40°C to –40°C, which corresponds to the exothermic peak representing the crystallisation of the gel. The heating phase is the rise from –40°C to 40°C, which corresponds to the heat absorption peak representing the melting of gel ice crystals[31].

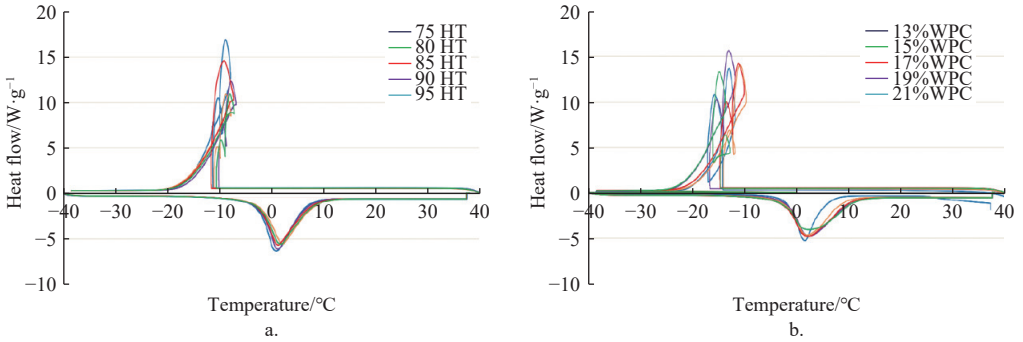


Figure 4 Effect of heat treatment on freeze-thaw stability test of DCF heat-induced gel

From Figure 4, the exothermic peak in the cooling phase was around 1°C and the heat absorption peak in the heating phase was around –10°C. The peak temperature decreased as the temperature increased. At 90°C, the peak showed an increasing and then decreasing trend, and the exothermic peak followed the same pattern. In Figure 4b, the peak DCF concentration trend was similar to the peak temperature trend. The exothermic peak was around 1°C during the cooling phase and the heat absorption peak was around –15°C during the heating phase. The peak corresponded to a gradual increase in temperature with increasing DCF concentration. At a concentration of 19%, the peak showed a turnaround. The turnaround may be related to the size of the particles in the gel, which became less stable as the particle size increased[32]. When the gel was heated at 90°C, the particle size in the gel changed from the previous pattern and this resulted in a sudden increase in the freeze-thaw stability of 90°C. This required further experiment to look at the microstructure of the observed gels, which was further tested below by CLSM.

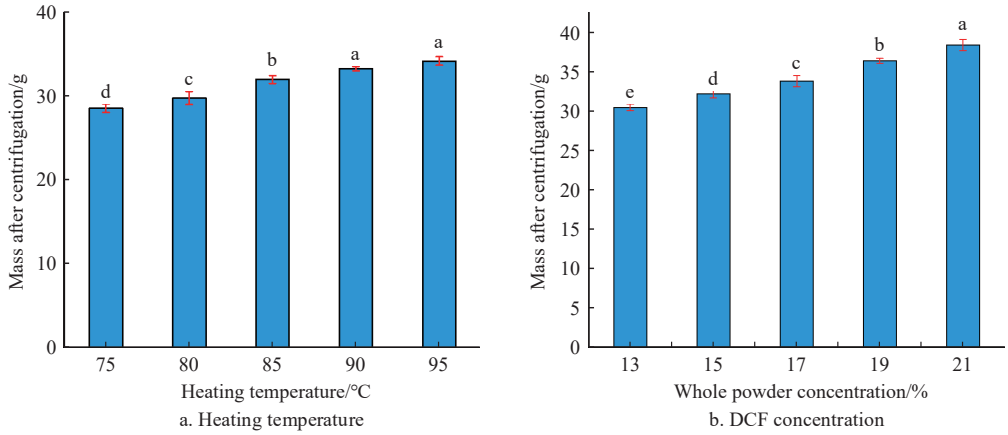
3.5 Water holding test results

Figure 5a shows the variation of water holding capacity of heat-induced gel with heating temperature. It can be seen from Figure 5a that the water holding capacity of the heat-induced gel increases significantly with the increase of the heating temperature (p<0.05). The water holding capacity of the heat induced gel when heated at 95°C was significantly higher than that at other heating temperatures. The enhancement of water holding capacity of heat-induced gel may be related to the degree of denaturation of the gel. As the heating temperature increased, the degree of gel denaturation increased significantly (p<0.05), which exposed more polar side chains and peptide bonds. As a result the gel had a greater ability to trap and retain water molecules[17].

Figure 5b presented the effect of DCF concentration on heat-induced gel water holding capacity. The water holding capacity of the prepared heat-induced gels increased significantly with the increase of DCF concentration (p<0.05). This may be due to the increased concentration of DCF, which led to enhanced interactions between the heat-induced gels, which contributed to the gel network structure more stable. Under the same high temperature (95°C) treatment, the DCF concentration was proportional to the degree of hydrophobic group exposure, network structural stability, and water retention[33].

3.6 Confocal microscopy tests results

Figures 6a-6e depict the effect of heating temperature on heat-induced gelation by confocal microscopy. From Figures 6a-6c, with the increase of temperature, the protein groups in the heat-induced gel gradually aggregated, and the volume gradually increased.



Note: Values in a column with different superscripts mean significantly different (p<0.05).

Figure 5 Effects of heating temperature and DCF concentration on water holding capacity of DCF heat-induced gel

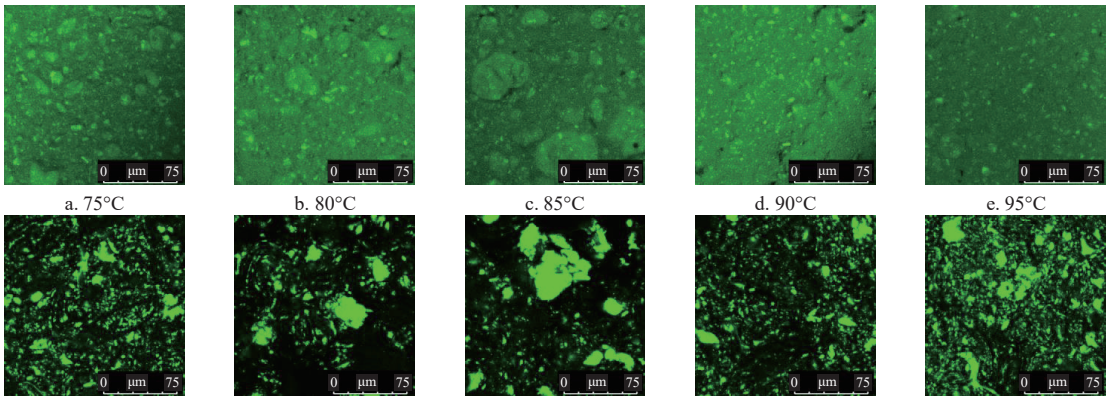


Figure 6 Heating temperatures and concentrations on heat-induced gel CLSM

At a heating temperature of 90°C in Figure 6d, the protein enrichment was broken into fine proteins and in high numbers. The number of proteins decreased significantly when heating was continued to 95°C in Figure 6e, which was consistent with the results of the DSC test. This may be due to the temperature altering the protein aggregation process, which was in agreement with the theory of secondary protein aggregation during gel formation. This may be due to an increase in the alpha-helix, which was related to the degree of protein aggregation and thought to be a typical feature of secondary changes in proteins affected by heat treatment, which was similar to the findings of Chen et al.[34]

Figures 6f-6j depict the effect of DCF concentration on heat-induced gelation by confocal microscopy. The protein exhibited the same phenomenon as the temperature, and the DCF concentration also affected the protein aggregation. In Figures 6f-6h, the proteins gradually aggregated and the protein groups became larger in size. In Figures 6i and 6j, the protein groups became finer and denser. This phenomenon was consistent with rheological testing, which was because larger protein aggregations were not conducive to gel formation[34].

4 Conclusions

This study focused on the effects of DCF concentration and heat treatment temperature on the functional properties of heat-induced gels from defatted chickpeas. The results showed that the heat-induced gel storage modulus of defatted chickpeas increased significantly after treatment, which in turn significantly improved the heat-induced gel viscoelasticity, resistance strength, and network structure stability. With increasing heating temperature and DCF concentration, the number of polar side chains, peptide bonds and hydrophobic groups of proteins in the heat-induced gels increased significantly, which enhanced the ability of the heat-induced gels to retain water molecules. From the microstructure, with the increase of the heat treatment temperature, the content of α-helix in the heat-induced gels protein gradually increased and then turned, which caused a similar change in the degree of protein aggregation. The protein clusters in the heat-induced gel were more uniform and smaller at a temperature of 95°C or a DFC concentration of 21%. The above results indicated that temperature and DCF concentration had significant effects on the functional properties of the defatted chickpea heat-induced gels.

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