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Effect of roasting and high-pressure homogenization on texture, rheology, and microstructure of walnut yogurt

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ABSTRACT

The effect of roasting and high-pressure homogenization on the quality of yogurt made from peeled walnut kernels was explored in this study. The G' and G'' values of yogurt made from walnuts roasted at high temperatures were reduced. The water-holding capacity and hardness of walnut yogurt were reduced to 47.73% and 24.22 g, respectively. Increasing the homogenization pressure reduced the particle size of the walnut yogurt to 20.50 µm. Homogenized walnut milk at 150 MPa increased the viscosity, hardness, and consistency of yogurt product from 11.71 to 16.74 Pa.s, from 30.01 to 71.63 g and from 283.17 to 455.24 g·s, respectively. The confocal laser scanning microscope observation demonstrated a reduction in the size of fat and protein micelles in the homogenized yogurt samples, resulting in a compact structure. This study will contribute valuable scientific insights to the advancement of plant-based yogurt quality.

1. Introduction

Yogurt is widely regarded as a healthy food by consumers (McKinley, 2005). However, traditional yogurt is not suitable for people suffering from milk protein malabsorption allergies, and other related health problems. As a result, a large number of people are seeking dairy alternatives. In recent years, plant-based fermented foods and beverages have received more attention due to their beneficial effects on health and improved sustainability of food production. Therefore, the development of plant-based yogurt is one of the alternative strategies to address these concerns and replace traditional yogurt (Jeske et al., 2018).

In the past, many plant-based yogurts with soybeans and oats as raw materials have been studied (Bruckner-Guhmann et al., 2019). Although these plant-based yogurt have been successfully produced, such products generally suffer from texture problems caused by phase separation, and when acidifying such plant-based systems, the instability of the proteins leads to the formation of a discontinuous and weak gel, which is still not as good as conventional yogurt (Bernat et al.,2014; Grasso et al., 2020). Walnut (*Juglans regia*) is a widely consumed nut crop that belongs to the genus *Juglans* and family Juglandaceae (Shi et al., 2018). Due to the high nutritional value of walnuts (including fats of 58.3–65.2%, proteins of 15.1–17.4%, carbohydrates of 13.9–19.4%, cellulose of 1.5–2%, minerals of 1.7–2%) (Ma et al., 2019), attempts have been made to use them in plant-based yogurts. Cui, et al. (2013) prepared fermented walnut milk with pure walnut milk beverage as a fermentation substrate and kefir grains as a starter. The results showed that walnuts had the potential to be fermented by lactic acid bacteria (the pH was 4.16, and the count of lactic acid bacteria was 1.1×10^8 CFU/mL). In addition, the structural instability of plant proteins caused by fermentation and acidification could lead to a weakened product texture and aqueous phase separation during storage after the beverage is prepared (Xu, et al., 2019).

The typical flavor and color of plant-based raw materials, like soybeans and walnuts, can be efficiently improved by roasting (Bagheri, et al., 2019). Navicha, et al. (2017) discovered that roasting at 110 °C for

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80 min helped inhibit lipoxygenase (LOX) activity in soybean, decrease the amount of volatile substances (like medium-chain aldehydes, medium-chain alcohols, and hydrogen peroxide derivatives), which successfully improve the flavor of soybean milk. Zhu, et al. (2020) found that both the number of aroma compounds and aroma intensity in walnut milk samples prepared by roasting walnuts increased significantly. This soybean milk showed a typical nutty, roasted flavour, with better flavour characteristics. Peng and Guo. (2015) discovered that the blanching of soybean at the temperature $\geq 70\ ^\circ C$ changed the compact and rigid texture of traditional soymilk gel and made it "yogurt-like". In addition, the Maillard reaction occurring during the heating process can promote the covalent cross-linking of proteins, increase the content of hydrophobic groups in the long chain of proteins, and enhance hydrophobic interactions. Thus, roasting can enhance the viscoelasticity as well as the texture of the plant-based yogurts and affect their enzyme activity and digestibility. The relatively stable gel structure gives the fermented walnut milk greater firmness, consistency, cohesiveness, and brightness (Zhang, et al., 2020). Furthermore, our group discovered that roasting walnuts not only enhanced the flavor of the fermented walnut milk, but the gel strength is also temperature-dependent (Fu et al., 2022). Therefore, more in-depth research is needed to determine whether roasting can improve the gel structure of walnut yogurt and how to improve it.

In the traditional yogurt industry, high-pressure homogenization (HPH) treatment is applied to make raw milk uniform before sterilization and fermentation steps. Generally, 10-20 MPa and 5 MPa are used as the first- and second- stage pressure respectively within the temperature range of 55-65 °C during HPH. In this way, the milk fat balls can be broken into smaller size and their specific surface areas are increased, which can prevent the creaming of yogurt during fermentation or storage. It also can reduce whey separation, enhance brightness, and improve the consistency of yogurt (Muramalla and Aryana, 2011). HPH is indispensable in the production of beverages containing plant proteins. Levy, et al. (2021) used 200 MPa HPH to produce a stable emulsion of potato protein isolate (PPI) and rapeseed oil and obtained like-yogurt gel by fermentation. They found that HPH could significantly enhance the brightness and water-holding capacity, which were important quality indicators of PPI yogurt substitutes. HPH is effective in improving the gel stability of plant-based yogurts while retaining the nutrients and flavor of the plant material. These changes were mainly attributed to the particle size changes caused by a comprehensive process of cavitation, turbulence, impacts, and shear forces (Serra, et al., 2007). Despite the application of HPH to enhance gel's texture properties, the effect of HPH on the acid coagulation process of walnut emulsion (a complex system rich in protein, fat, carbohydrate, and so on) has not been reported.

The purpose of this study was to combine roasting and high-pressure homogenization technology to solve the shortcomings of present plant-based yogurts. We prepared walnut milk from roasted or unroasted walnuts and homogenized it at different pressures to produce walnut yogurt. The textural and rheological properties of different types of walnut yogurt were compared, and the effects of roasting and HPH on the fermentation process and the gel characteristics of walnut yogurt were explored. This study will provide theoretical and data support for the development of a plant-based yogurt sector.

2. Materials and methods

2.1. Materials

A commercially available peeled walnut kernel was purchased from Changsheng Agricultural Products Trading Co., Ltd. (Shaanxi, China) with about 15% protein, 70% fat, 2% ash, and 2% crude polysaccharides (To be clarified, the peeled walnuts are prepared by first soaking the kernels in water for 6 h, during which some skin is naturally detached; the walnuts are then rinsed thoroughly, with deeper cleaning and skin removal being achieved using a high-pressure water gun; subsequently, the walnuts are transferred to a picking table where debris, damaged, and spoiled nuts are removed; the drying process consists of two phases: initially, the walnuts are baked at 40-50 °C for 7-8 h to remove excess moisture, followed by one further hour at 80-90 °C for additional drying). Freeze-dried yogurt starter cultures obtained from Chr. Hansen (YF-Mild 1.0, Horsholm, Denmark) consist of *Streptococcus thermophilus and Lactobacillus delbruekii* subsp. *Bulgaricus*. Diacetyl tartaric acid ester of mono(di)glycerides (DATEM), pectin, gellan gum, sucrose, and glucose were purchased from Cuifeng Technology Co., Ltd. (Beijing, China). Walnut oil (Shandong Liangjun Yiyou Machinery Co., Ltd, QYZ-230, China) is obtained by pressing walnut kernels at 13 MPa for 30 min using an automatic hydraulic oil press.

2.2. Walnut yogurt preparation

The walnut kernels were divided into two parts, one of which was roasted at 152 °C for 15 min and the other was not, to obtain roasted walnut kernels (RWK) and non-roasted walnut kernels (NWK), respectively. Two different raw walnut materials were squeezed to obtain defatted walnuts with about 35% protein, 38% fat, 5% ash, and 5% crude polysaccharides. These two kinds of walnut meals were respectively soaked in deionized water with a 1:8 mass ratio and ground by a colloid mill (General Machinery Manufa, JMS-80, China), according to the following formula: 4% sucrose (w/w), 3% glucose (w/w), 0.2% DATEM (w/w), 0.07% pectin (w/w), 0.05% gellan gum (w/w), and 1% walnut oil (w/w) (this oil is obtained by pressing roasted walnut (165 °C,15 min) for the purpose to enhance the flavor of walnut yogurt). And then the walnut crude milk was homogenized two-cycle at 0, 30, 60, 90, 120, and 150 MPa using a laboratory homogenizer (ATS Engineering Limited, AH-1300, China) to obtain roasted walnut milk (RWM) and non-roasted walnut milk (NRWM), respectively. The obtained walnut milk was heated at 90 °C for 15 min for sterilization and then stored at 4 °C.

The commercial starter YF Mild 1.0 (including *Streptococcus thermophilus* and *Lactobacillus bulgaricus*) was diluted in a 10-fold gradient to ferment the different types of walnut milk mentioned above at a final inoculum of 0.02% (V/V), incubated at 40 °C for up to 11 h to obtain a roasted walnut yogurt (RWY) and a non-roasted walnut yogurt (NRWY). Then, the yogurt was stored and ripened at 4 °C for 24 h for subsequent analysis.

2.3. Determination of titratable acidity and pH

The changes in pH and titratable acidity of walnut yogurts were measured at 1 h intervals during 0-11 h fermentation. All samples were measured twice. Titratable acidity was measured by an automatic potentiometric titrator (907 Titrando, Metrohm, Switzerland). Yogurt samples (10 g) were put into a flask, along with 20 mL of deionized water, and mixed well. KOH solution (0.1 M) was used for the potentiometric titration, and pH 8.3 was used as the endpoint. Nitrogen was blown into the bottle during the titration to prevent the solution from absorbing CO_2 from the air. The volume of KOH consumed was recorded and brought to the following equation for calculation.

$$X = \frac{C \times (V - V_0) \times 100}{m \times 0.1} \tag{1}$$

where X was titratable acidity (°T), C was the molar concentration of the KOH standard solution (mol/L), V was the volume of KOH consumed during titration (mL), V_0 was the volume of KOH standard solution consumed in the blank experiment (mL), and m was the sample mass (g).

The pH values of different yogurt samples were measured at room temperature using a pH meter (Seven Compact, Mettler Toledo, Switzerland).

2.4. Determination of microrheological properties of walnut yogurt during fermentation

The micro rheometer properties of different walnut milks were measured during 0-3 h of fermentation and coagulation stage by a micro rheometer (Rheolaser Master, Formulaction, France). This time setting is due to the reason that pH values of fermented walnut milks are close to the isoelectric point of walnut proteins 4.8 after 3 h fermentation.

After inoculation, 20 mL of walnut milk was inserted into the sample cell of the micro rheometer, and the sample pool was inserted into the micro rheometer. The changes in macroscopic viscosity index (MVI), elastic index (EI), solid–liquid balance (SLB), and fluidity index (FI) in the fermentation process were monitored at 40 °C. Data was collected every 3 min and measured for 3 h.

2.5. Rheological properties analysis

2.5.1. Viscosity

The experiment conditions refer to a study by our group (Jiao, et al., 2018) with some modifications. The walnut yogurt was churned clockwise five times and then counterclockwise five times. Then it was used to determine its apparent viscosity using a DHR-2 rheometer equipped with an aluminum plate (40 mm diameter, 1 mm gap). The temperature was controlled at 25 ± 1 °C, and the shear rate ranged from 0.1 to 100 s^{-1}. The data were collected in a point-wise, logarithmic manner.

The resulting data were fitted using a Herschel-Bulkley model, which can be expressed by the following equation:

$$\tau = \tau_0 + K\gamma^n \tag{2}$$

where τ was the shear stress (Pa), τ0 was the yield stress (Pa), K was the consistency coefficient ($Pa \cdot s^n$); γ was the shear rate (s^{-1}) and n was the flow behavior index.

2.5.2. Frequency sweep

The experiment conditions refer to a study by our group (Li, et al., 2023) with some modifications. Prior to the formal experiments, all samples were subjected to strain sweeps at a constant frequency of 1 Hz to determine their linear viscoelastic region. Subsequently, the temperature was controlled to be constant at 25 ± 1 °C, the strain amplitude was set at 0.1% (within the linear viscoelastic region), and frequency sweep were performed at 0.1-10 Hz to determine the viscoelastic properties of different yogurts.

The resulting data were fitted using a Power-Law model, which can be expressed by the following equation:

$$G' = k' \bullet \omega^{n'} \tag{3}$$

$$G'' = k'' \bullet \omega^{n''} \tag{4}$$

where G' was elastic modulus (Pa), G" was viscous modulus (Pa), ω was the angular frequency (rad/s), k' (k") was the power-law constant, and n' (n") was the frequency index.

2.6. Particle size analysis

The particle size of the yogurt sample was measured by a laser particle sizer (Mastersizer 3000, Malvern, England) equipped with an automatic dispersion device (Hydro EV, Malvern, England). The relevant parameters were as follows: the particle refractive index was 1.520, the particle absorption rate was 0.1, the dispersant was water, and the refractive index of the dispersant was 1.330. The volume-weighted average diameter (D[4,3]) and particle size distribution were obtained from the instrument software. All samples were measured in triplicate.

2.7. Lightness measurement

The average of the L* values (luminance from 0 to 100) of walnut yogurt was recorded using a spectrophotometric colorimeter (CS-580A, Kemper Technology Co., Ltd., China), which was calibrated using a black-and-white standard tile (standard light source D3 and 65° viewer), and the samples were placed in a glass that was not exposed to external light. Experiments (5 treatments) were performed in 3 independent replications, and results are presented as the mean of triplicates.

2.8. Texture analysis

The hardness and consistency of yogurt samples were measured by a texture analyzer (TA.XT plus, Stable Micro Systems, England) equipped with an A/BE-40 mm probe. The pre-test speed, test speed and post-test speed are 1 mm/s, 1 mm/s and 10 mm/s, respectively. The strain is 30% and the trigger force is 10 g.

2.9. Water-holding capacity (WHC)

The experiment conditions refer to a study by Wang Qiang's group (He et al., 2014) with some modifications.. The yogurt sample (10 g) was loaded into a centrifuge tube and then centrifuged at 1465 g, 4 °C for 15 min. The supernatant was poured out, and the centrifuge tube was inverted onto a filter paper for 10 min before being weighed separately. The weight of the sediment in the tube was weighed, and the water-holding capacity of the yogurt was calculated using the following formula:

$$\text{WHC}\% = \frac{W_1}{W_2} \times 100\% \tag{5}$$

where W_1 was the sediment weight, W_2 was the sample weight.

2.10. Microstructure analysis

The experiment conditions refer to a study by Li, et al., (2022) with some modifications. A confocal laser scanning microscope (CLSM, TCS-SFIG. 6, Leica Microsystems Inc., Germany) was used to observe the microstructure of walnut yogurt samples, which were refrigerated at 4 °C for 1 day after fermentation. One milliliter sample was taken on a viewing dish, and 20 μL of Nile Red (0.1 mg/mL isopropanol) was used to fluorescently label the fat with an excitation wavelength of 488 nm, and the signal color was set to red; 20 μL of Nile Blue (0.1 mg/mL in isopropanol) was used to fluorescently label the protein with an excitation wavelength of 633 nm to set the signal color to green. The dyed sample was left for 30 min in the dark at 4 °C before observation.

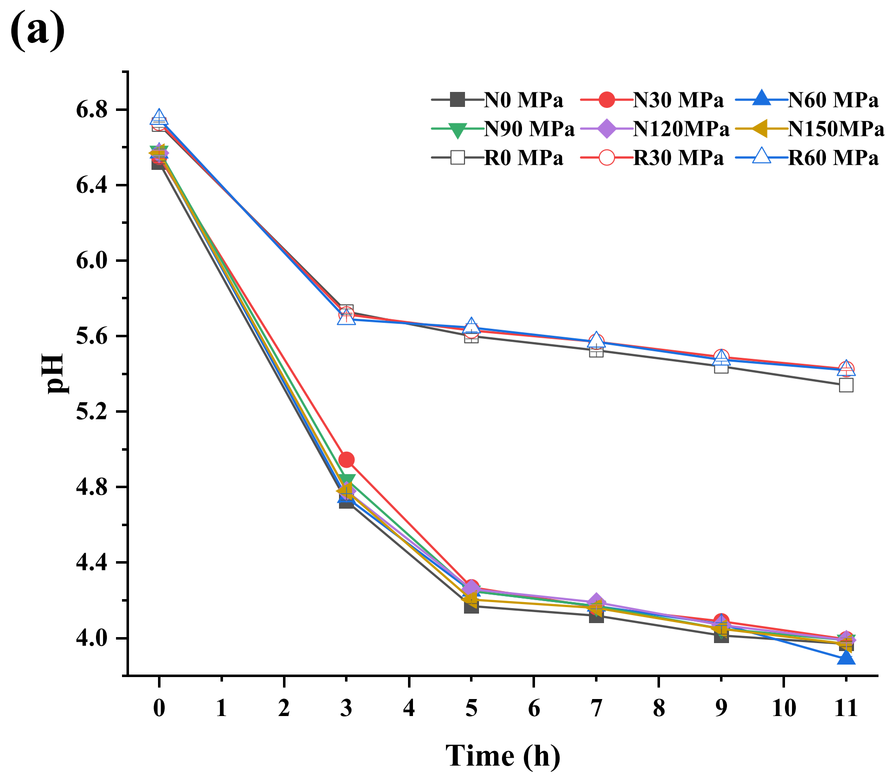
2.11. Statistical analysis

One-way analysis of variance (ANOVA) was performed using SPSS 19.0, and all data were presented as "mean ± standard deviation (SD)". Significance ($p < 0.05$) was determined using Duncan's multiple-range tests.

3. Results and discussion

3.1. Effect of roasting and HPH on the pH, titratable acidity, and coagulation process of walnut yogurt

Previous studies showed that lactic acid bacteria could use carbohydrates (from plant milk or additionally added monosaccharides, disaccharides, and oligosaccharides) to grow, multiply, and metabolize acid production (Tangyu et al., 2019). The protein in the plant milk became acid-coagulated when the plant milk pH was near the isoelectric point of the protein, resulting in a yogurt-like texture. In this study, all



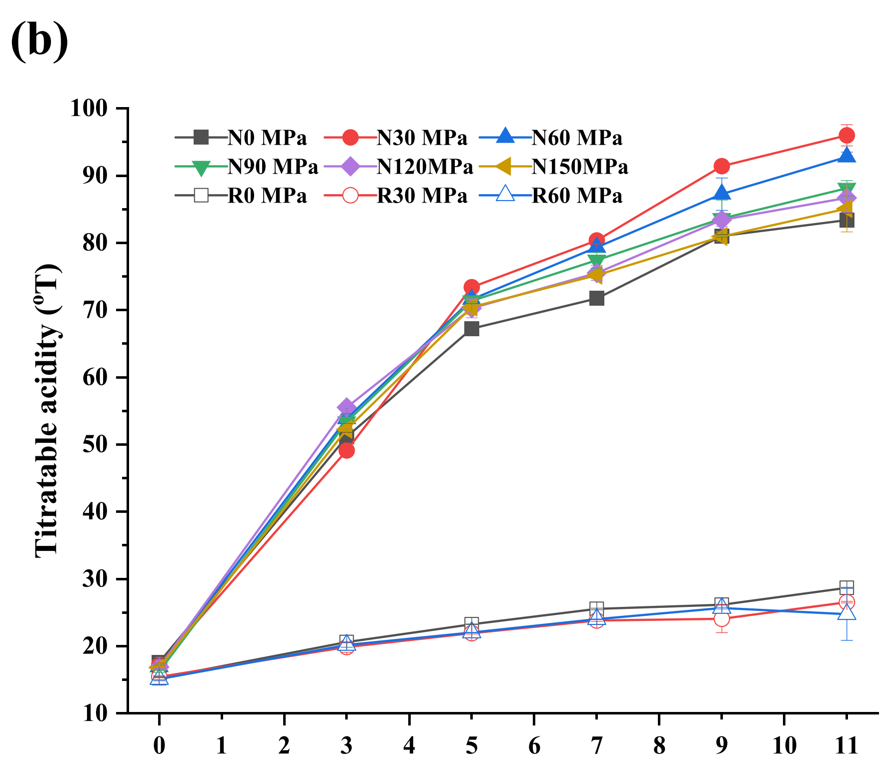
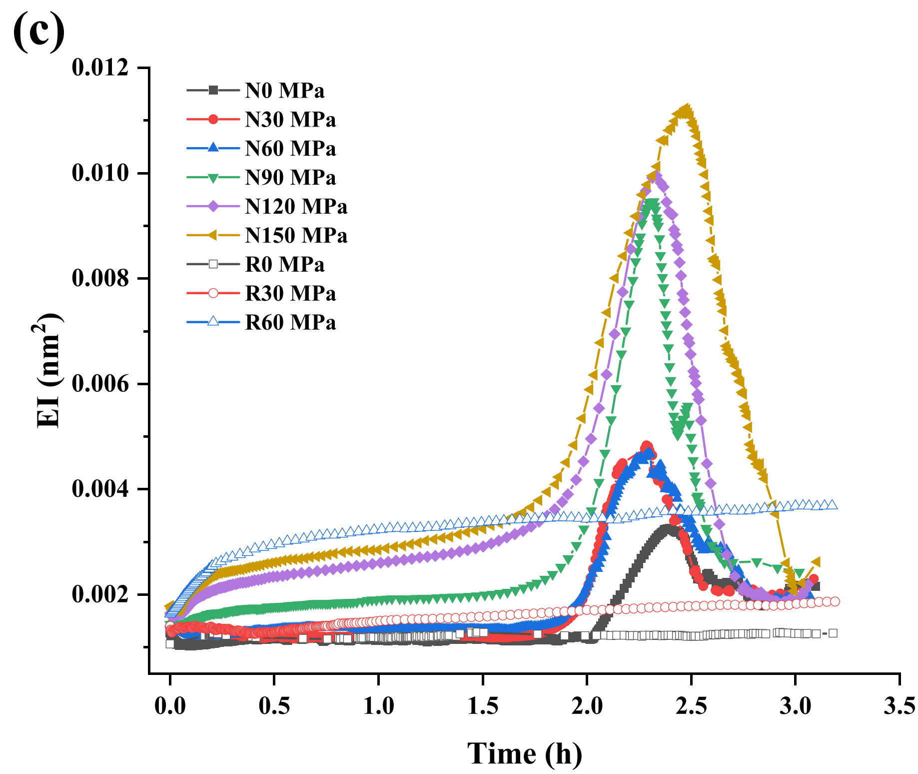


Fig. 1. Fermentation profiles of roasting (R) and non-roasting (N) walnut to produce yogurt at different homogenization pressures (0–150 MPa): (a) pH; (b) Titratable acidity;(c) Elastic index; (d) Macroscopic viscosity index; (e) Solid-liquid balance; (f) Fluidity index.



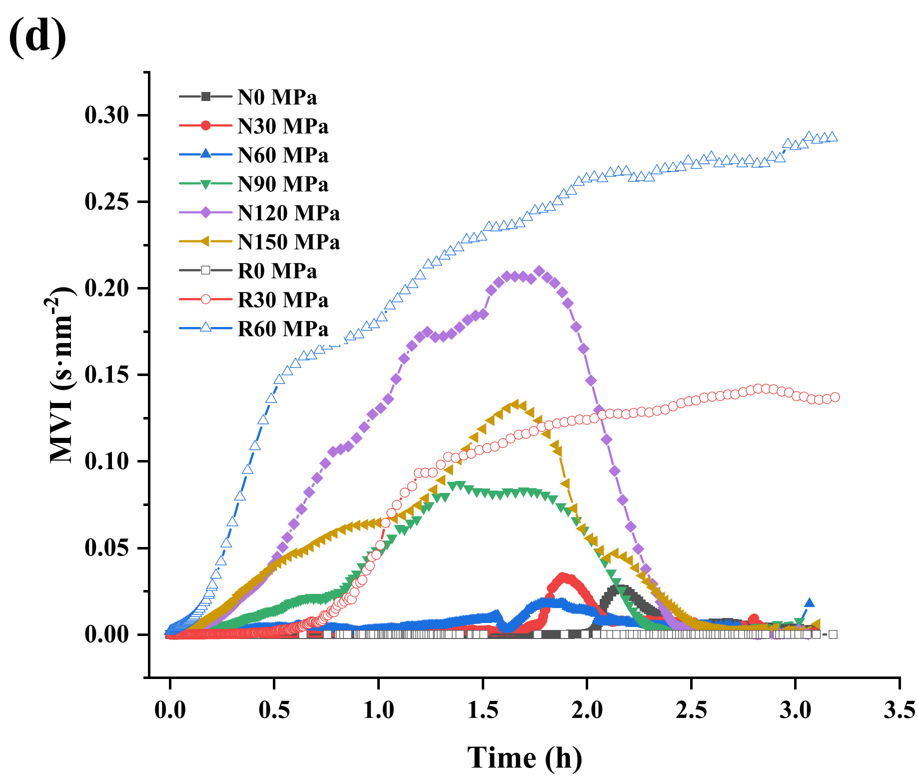
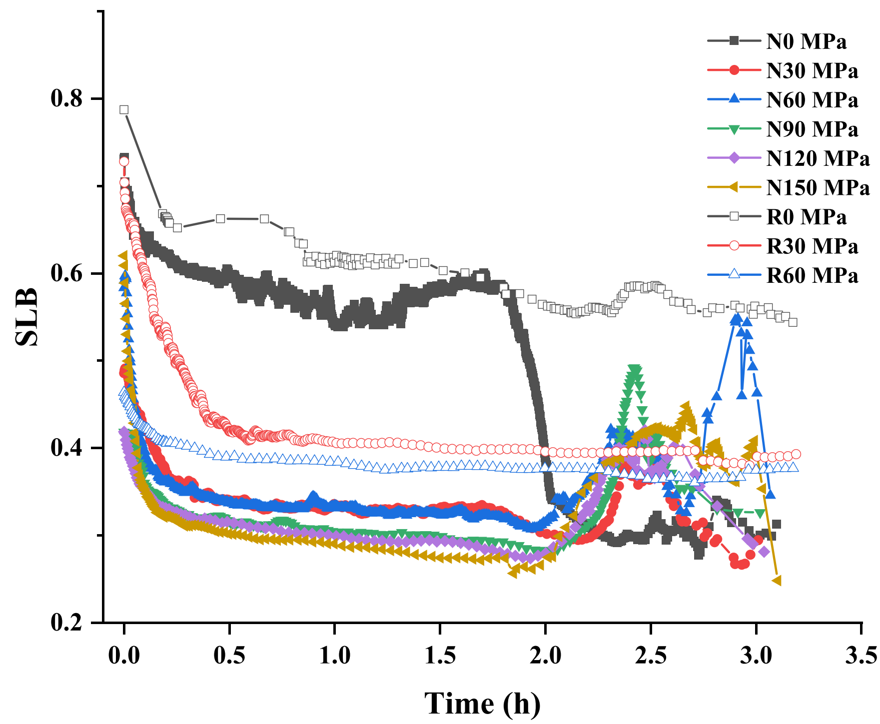


Fig. 1. (*continued*).

walnut milk samples were additionally supplemented with the same amounts of sucrose and glucose. Thus, microorganisms could utilize these carbon sources to produce lactic acid, increasing the acidity of the yogurt and decreasing its pH. The changes in acidity and pH during the fermentation of different walnut yogurt samples were examined (Fig. 1 (a)). In walnut yogurt, the pH decreased to near the isoelectric point (pH = 4.8) of walnut protein (Mao et al., 2014) at approximately 3 h of fermentation and then leveled off gradually, exhibiting a fermentation profile similar to that of traditional animal-based yogurt (Parnell-Clunies et al., 1988). As shown in (Fig. 1(a)), roasting treatment

(e)



(f)

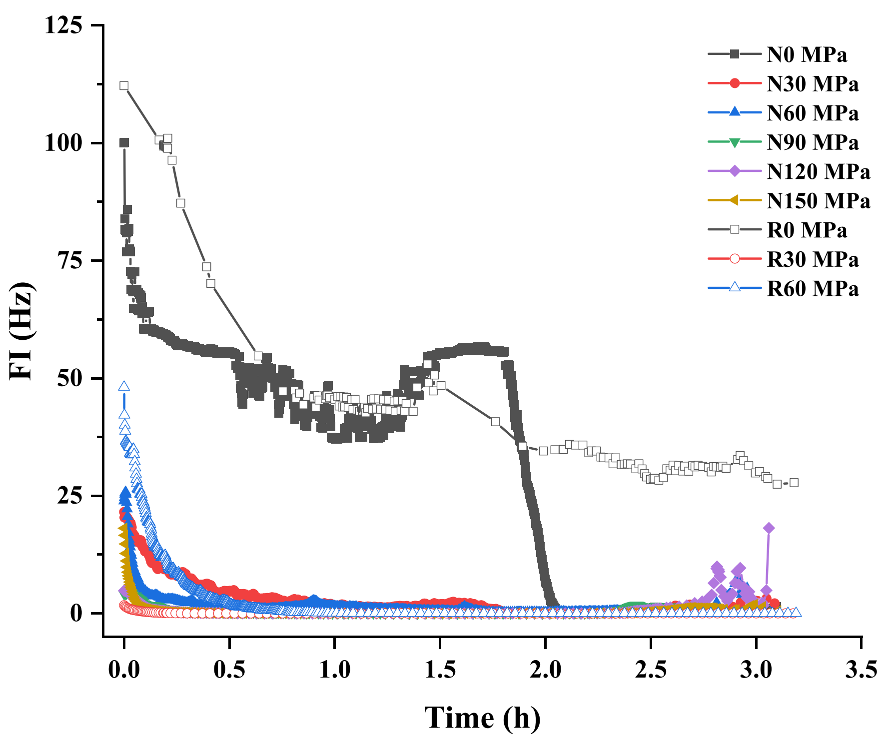


Fig. 1. (*continued*).

significantly inhibited walnut yogurt fermentation, and all RWY samples had a significantly greater pH at the end of fermentation than NRWY ($p < 0.05$). It was probably because the microorganisms had not been domesticated and they were not well adapted to the environment of roasted walnut milk, resulting in a slower acid production rate. In a complex food system such as yogurt, homogenization had less influence on yogurt pH (Fig. 1(a)). As shown in (Fig. 1(b)), the acidity of the non-homogenized walnut yogurt at the end of fermentation was 83.40 ±

1.03 °T, which was significantly lower than that of the walnut yogurt, 96.01 ± 1.58 °T, after homogenization by 30 MPa ($p < 0.05$). It might be due to the fact that homogeneous treatment partially unfolds the protein structure, which is more favorable for the degradation of the defatted-walnut protein polypeptide chains by lactic acid bacteria (Sharma et al., 2023).

MS-DWS was a microrheological technique to study the gelation mechanism of soft matter such as yogurt. Without destroying the samples, the rheological properties were investigated by monitoring the movement of the particles within the samples (Rohart et al., 2016). The changes in rheological characteristics for all samples are shown in (Fig. 1 (c-f)).

The EI correlates with the solid-like behavior of yogurt and with the storage modulus at small deformation rheology, so it could be used as an indicator of gel stiffness (Zhang et al., 2021). The elastic changes in the gel formation process could be divided into 3 stages for non-roasted yogurt (Fig. 1(c)). Combined with (Fig. 1(a)), it could be concluded that during the first 2 h of fermentation, the pH value of the sample was reduced to 5.4 from the initial value of 6.6 and the sample was in a low elastic state. After that, at 2-2.5 h of fermentation, the pH of samples decreased from 5.4 to 4.8, and walnut protein began to coagulate and show a rapid increase in elasticity. When the pH decreased to the walnut protein isoelectric point at pH = 4.8, the EI values of samples treated with different homogenization pressures all reached a peak. The EI peak value 0.01123 nm^2 was maximal when the homogenization pressure was 150 MPa. The hydrophobic interactions were the main force for the formation of the gel network, and the stiffness of the obtained gels increased as the hydrophobic interactions of the proteins increased with the homogenization pressure (Cruz et al., 2009). Finally, as the fermentation proceeded, the pH value continued to decrease and moved away from the isoelectric point of the walnut protein. Hence, the protein solubility enhanced, the gel strength weakened, and the EI value also decreased gradually, which was consistent with the conclusion of Bai, et al., (2020). MVI values had a similar trend to EI values (Fig. 1(d)). For RWY, the pH of the walnut protein did not reached its isoelectric point (Fig. 1(a)), so the change in elasticity and stickiness did not have a peak, but the increase in homogenization pressure contributed equally to the increase in viscoelasticity of RWY.

The SLB (Fig. 1(e)) showed the time course of the sample's solid-liquid properties. When SLB = 0, the sample was pure elastic/solid; 0 < SLB < 0.5, the sample was predominantly elastic/solid; 0.5 < SLB < 1, the sample was predominantly viscous/liquid; SLB = 1, the sample was pure viscous/liquid (Luo et al., 2020). For the NRWY, the homogenized walnut milk, which can be regarded as a walnut emulsion system, quickly decreased SLB values from 0.6 to 0.3 after initiating gelation (around 2 h), representing a rapid transition of the samples from the liquid to the solid state. Whereas the unroasted walnut emulsion without homogenization consistently had a solid-liquid equilibrium value > 0.5 since no coagulation occurred. All samples after homogenization by 30-150 MPa rapidly decreased their SLB values below 0.5 in the early stages of fermentation, indicating that walnut emulsion (a complex system containing polysaccharides, proteins, and oils) can have some viscoelastic properties after HPH. The higher the homogenization pressure, the lower the SLB value. After that, as the sample pH continued to decrease, the dehydration condensation continued to strengthen, and syneresis occurred. Thus, the solid-liquid equilibrium value of all samples fluctuated. FI can reflect the speed of particle movement in the sample (Fig. 1(f)). The larger the FI value, the faster the particle movement and the worse the stability of the sample. The FI values of samples without homogenization before gelation were higher than that of homogenized samples. They decreased rapidly after coagulation started and remained stable.

3.2. Effects of roasting and HPH on rheological properties of walnut yogurt

3.2.1. Viscosity

Apparent viscosity was an important parameter affecting the sensory quality of yogurt. Some studies related to yogurt have been focused on rheological parameters and sensory properties that devote the additional quality characteristics of the yogurt (Icier et al., 2015). In the present study, the effect of homogenization treatment to the walnut crude milk on the rheological behavior of the yogurt was investigated. The apparent viscosity of the walnut yogurt samples decreased as the rate of shear increased. (Fig. 2(a)), which is in line with the previous study by Mei et al. (2017). When the shear rate was small, the apparent viscosity of yogurt was usually high. Afterward, viscosity rapidly reduced with increasing shear rate, proving that all samples were pseudoplastic fluids with shear thinning phenomena. This might be due to the destruction of the protein network and the rearrangement of protein aggregates and oil droplets with the increase in shear rate.

The rheological parameters of walnut yogurt were analyzed by the Herschel-Bulkley model. As shown in Table 1, the R^2 values of all samples were greater than 0.95, indicating that the Herschel-Bulkley model could better fit the rheological properties of walnut yogurt. All samples had significant differences in K value and n value ($p < 0.05$). The larger the K value, the smaller the n value, indicating that the consistency of the yogurt was greater. After roasting, walnut protein appeared denatured and thermally aggregated (Sorgentini et al., 1995). Because the cross-linking effect of walnut protein micelles was reduced, the final yogurt gel had a loose structure, so the roasting samples had relatively low K values ($\leq$0.75 Pa·s^n) and relatively high n values ($\geq$0.79). In addition, yield stress also increased from 2.25 Pa of N0 to 17.65 Pa of N150, and the RWY group's $\tau 0$ values were lower than the NRWY group. The greater the yield stress, the greater the shear force required for the sample to start flowing. Hence, the stronger the sample's ability to resist shear, the more its sensory performance was "harder" (Ciron et al., 2011). Therefore, the RWY group and N0 were softer in that sense.

3.2.2. Frequency sweep

The stress generated by deformation was elastic energy stored in the sample. It was a measure of the elasticity of the sample, expressed by the storage modulus (G'). The part of energy lost in the sample could be used to measure the viscosity characteristics of the sample, expressed by the loss modulus (G'') (Jiao et al., 2018). In the frequency range of 0.1-10 Hz, with the increase in scanning frequency, the values of G' and G'' of all samples showed an increasing trend. Furthermore, G' were higher than G'', indicating that the elastic components in the samples were dominant and showed the characteristics of being solid-like (Fig. 2(b, c)). In all samples, the G' and G'' of N150 were the highest. In addition, the values of G' and G'' gradually decreased with the decrease in homogenization pressure. In addition, the values of G' and G'' of yogurt in the RWY group were lower than those in the NRWY group, indicating that the gel structure of yogurt in the RWY was looser.

The experimental data were fitted using the Power-Law equation (Table 2). The variation trends of G' and G'' were consistent with the power-law parameters k' and k'', which significantly ($p < 0.05$) increased with increasing homogenization pressure. It indicated that the viscoelastic properties of yogurt increased with increasing homogenization pressure. Levy et al. (2021) used different homogenous pressure treatments of isolated potato proteins to make plant-based yogurt and reached the same conclusion. In addition, both k' and k'' of NRWY were significantly greater than RWY ($p < 0.05$), which might be explained by two aspects. On the one hand, all RWY samples were poorly fermented. The system pH could not reach the isoelectric point of the walnut protein at the end of fermentation and failed to form a stable yogurt gel structure. On the other hand, walnut proteins were partially denatured after roasting and easily formed precipitates after milk preparation. These

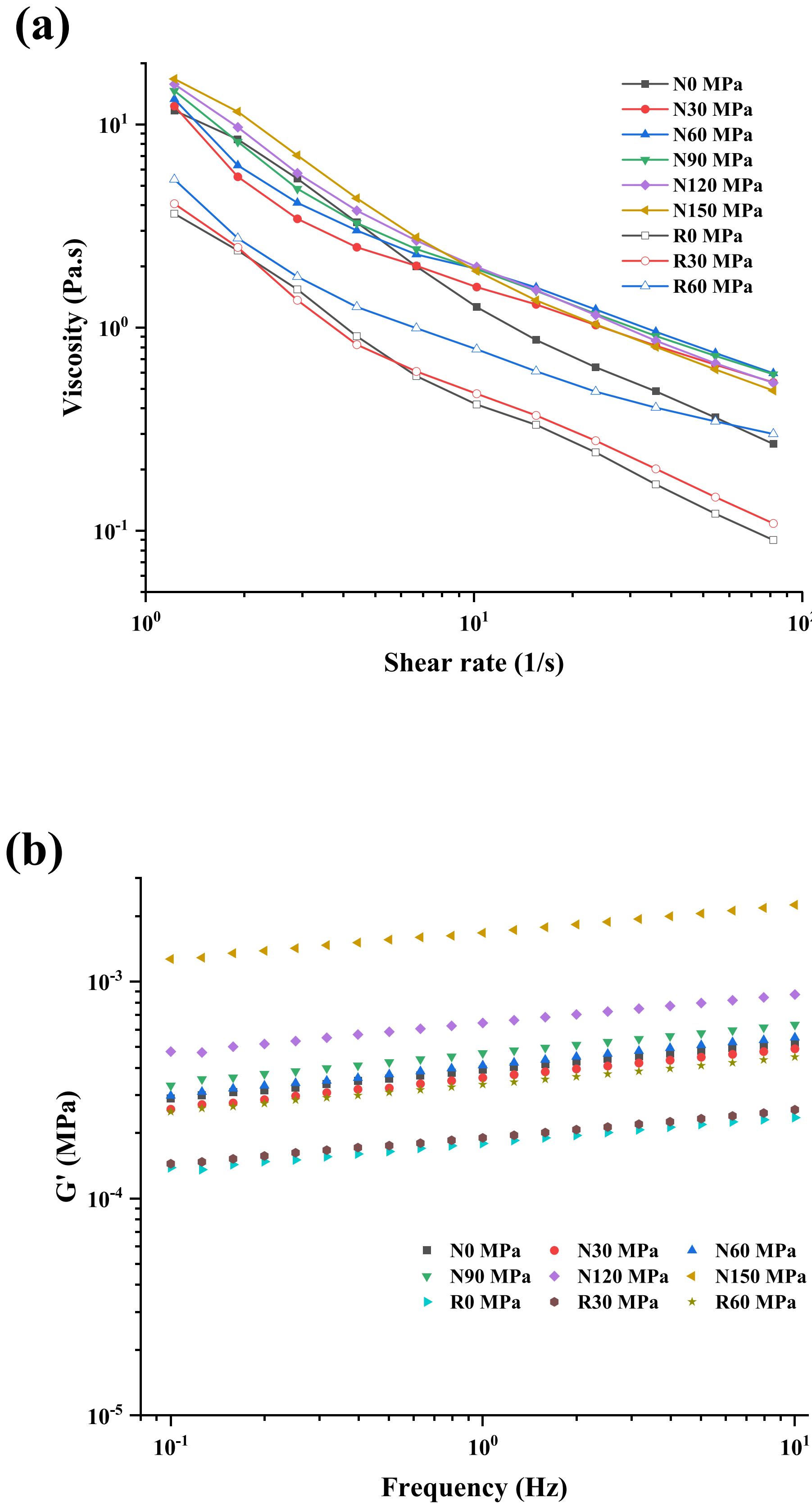


Fig. 2. Changes in apparent viscosity and frequency sweep curve of yogurt made from roasting (R) and non-roasting (N) walnut under different homogenization pressure (0–150 MPa). (a) viscosity; (b) elastic modulus; (c)viscous modulus.

(c)

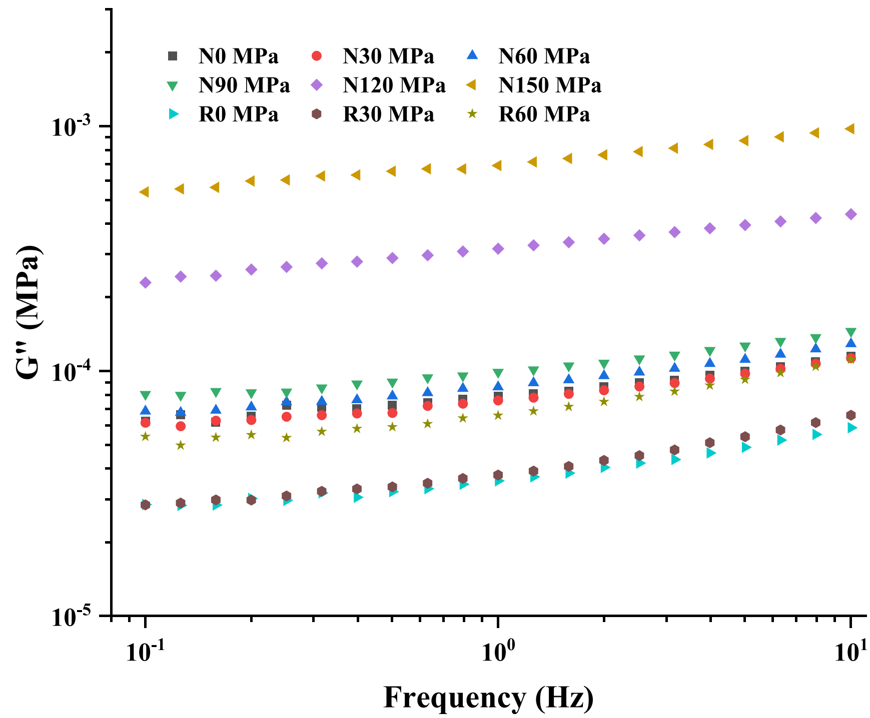


Fig. 2. (*continued*).

Table 1
Rheological parameters of yogurt made from roasting (R) and non-roasting (N) walnut under different homogenization pressures (0–150 MPa).

Parameter	N0	N30	N60	N90	N120	N150	R0	R30	R60
τ_0 (Pa)	2.25 ± 0.04^{d}	3.48 ± 0.64^{d}	7.21 ± 1.97^{c}	11.74 ± 2.64^{b}	12.15 ± 0.21^{b}	17.65 ± 1.91^{a}	1.09 ± 0.23^{d}	1.41 ± 0.89^{d}	2.08 ± 0.19^{d}
K (Pa·s^n)	1.20 ± 0.17^{e}	1.54 ± 0.22^{de}	2.14 ± 0.51^{d}	3.30 ± 0.16^{c}	4.22 ± 0.36^{b}	6.02 ± 0.58^{a}	0.21 ± 0.02^{f}	0.30 ± 0.08^{f}	0.75 ± 0.51^{ef}
n	0.62 ± 0.06^{c}	0.57 ± 0.01^{cd}	0.52 ± 0.03^{cd}	0.47 ± 0.01^{de}	0.36 ± 0.06^{e}	0.34 ± 0.02^{e}	0.99 ± 0.07^{a}	0.91 ± 0.01^{ab}	0.79 ± 0.13^{b}
R^2	0.980	0.997	0.999	0.999	0.998	0.994	0.957	0.966	0.983

Data are expressed as means ± SD. Different superscript characters (a-c) indicate significant ($p < 0.05$) differences in the same row.
τ0 (Pa) is the yield stress.
K (Pa·s^n) is the consistency coefficient.
n (dimensionless) is the flow behavior index.

Table 2
Power law model fitting of frequency sweep of yogurt made from roasting (R) and non-roasting (N) walnut under different homogenization pressures (0–150 MPa).

Parameter	N0	N30	N60	N90	N120	N150	R0	R30	R60
k' (Pa)	278.01 ± 57.58^{cd}	308.10 ± 21.21^{cd}	319.72 ± 98.90^{cd}	362.44 ± 67.88^{bc}	500.4 ± 32.06^{b}	1341.95 ± 205.34^{a}	224.03 ± 73.56^{cd}	152.52 ± 28.56^{d}	143.41 ± 42.21^{d}
n'	0.139 ± 0.007^{a}	0.129 ± 0.003^{bc}	0.134 ± 0.002^{ab}	0.136 ± 0.004^{a}	0.135 ± 0.001^{ab}	0.124 ± 0.003^{c}	0.124 ± 0.002^{c}	0.123 ± 0.001^{c}	0.123 ± 0.003^{c}
k'' (Pa)	60.79 ± 11.55^{cde}	64.20 ± 4.09^{cd}	68.75 ± 20.66^{cd}	79.71 ± 12.99^{bc}	109.37 ± 8.41^{b}	271.94 ± 38.61^{a}	43.10 ± 13.89^{de}	28.50 ± 5.36^{e}	27.96 ± 7.02^{e}
n''	0.135 ± 0.002^{cd}	0.127 ± 0.002^{d}	0.140 ± 0.005^{c}	0.132 ± 0.003^{cd}	0.129 ± 0.003^{d}	0.132 ± 0.004^{cd}	0.167 ± 0.010^{b}	0.181 ± 0.008^{a}	0.158 ± 0.001^{b}

Data are expressed as means ± SD. Different superscript characters (a-c) indicate significant ($p < 0.05$) differences in the same row.
k', k'' (Pa) is the power law constant.
n', n'' (Pa) is the frequency exponent.

precipitated protein aggregates did not allow protein molecules to form colloidal molecular masses with one another. And thus, after microbial fermentation and acid production, the hydrated protein network and gel structure were also weak (Nie, et al., 2022).

3.3. Effect of roasting and HPH on the size distribution, lightness, and CLSM of walnut yogurt

When homogenizing by high pressure, materials would simultaneously suffer from mechanical forces such as high-speed shearing, high-frequency concussion, cavitation, and convection impingement, thus forming broken structures. The effects of roasting and high-pressure homogenization on the particle size of walnut milk were shown in Fig. 3(a) and (b). The yogurt particle size decreased significantly ($p < 0.05$) as the homogenization pressure increased gradually (Fig. 3(a)). When yogurt was created with emulsions homogenized at the same pressure, the particle sizes of the RWY groups were all significantly smaller than the NRWY groups ($p < 0.05$). It was probably due to the different pH at the end of fermentation, which induced different degrees of protein aggregation. The PSD curve of yogurt shifted significantly

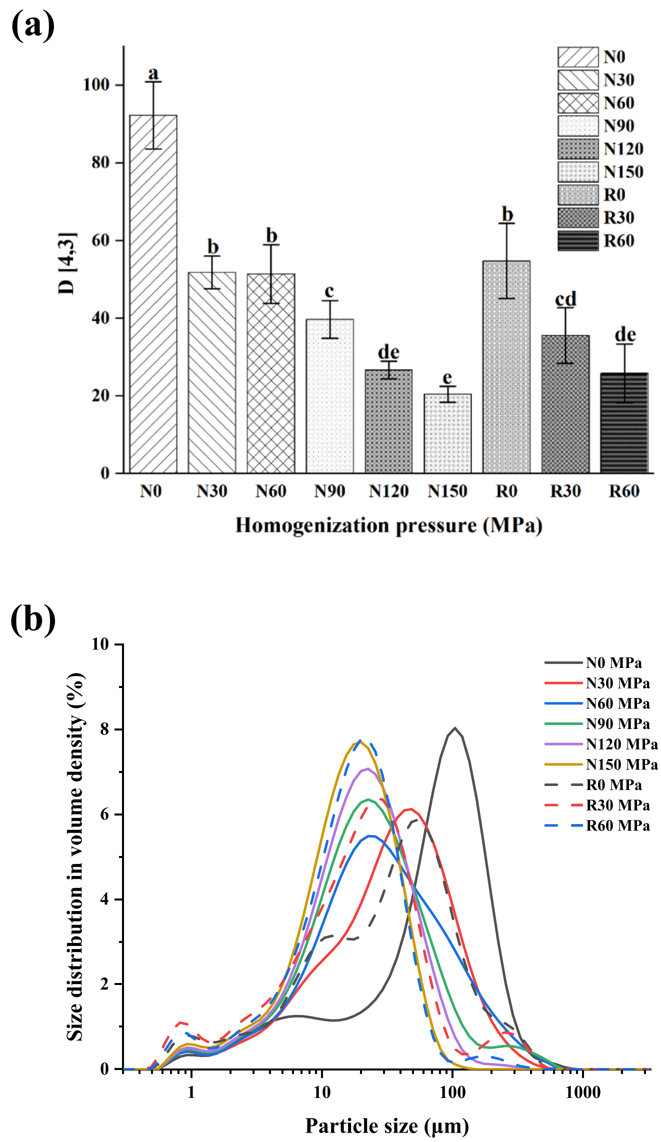


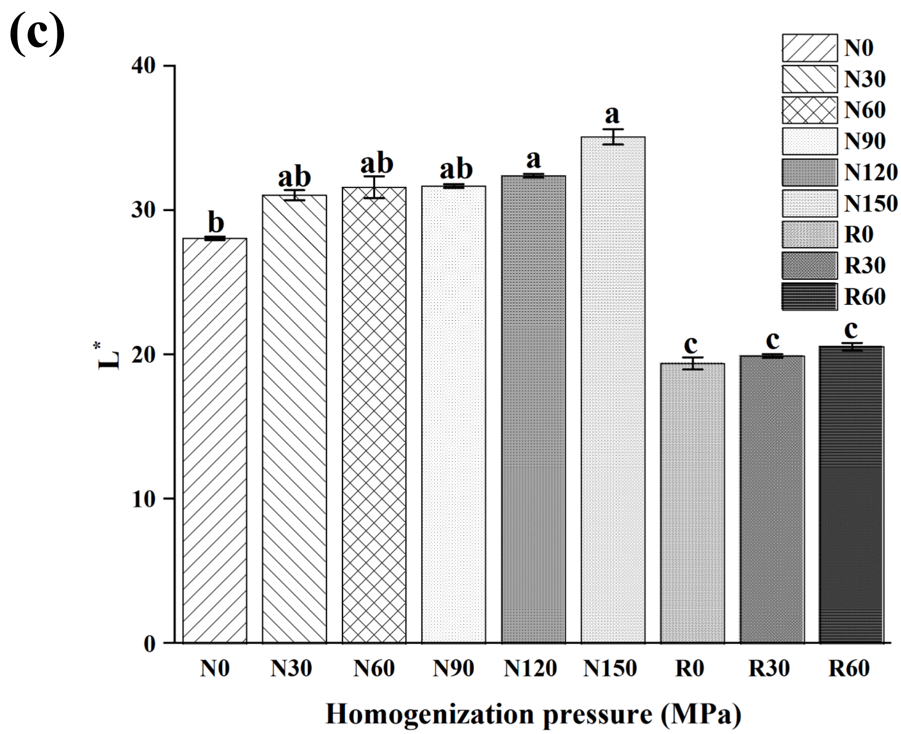
Fig. 3. Particle size, lightness and CLSM of roasting (R) and non-roasting (N) walnut to make yogurt at different homogenization pressures (0-150 MPa). (a) Mean particle size (D [4,3]); (b) Particle size distribution; (c) Lightness values; (d) CLSM (The scale bar represented 75 μm. Where green was protein and red was fat globule.). Values with different letters were significantly different ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

toward smaller particles as the homogenous pressure increased, while still maintaining a bimodal distribution (Fig. 3(b)).

The appearance of a food product was critical to consumer acceptance, and the lightness value was related to the light-reflecting and transmitting capabilities of an object. The lightness value of the yogurt made from emulsions homogenized at 150 MPa reached the highest 35.1 (Fig. 3(c)). Combined with the results of the particle size distribution, it could be speculated that the yogurt particle size was significantly reduced and the gel structure was denser and more uniform after high-pressure homogenization. Thus, the reflection of light on the yogurt colloids was enhanced, and the result was consistent with Hernandez and Harte (2008). On the contrary, walnut yogurt produces a looser gel structure in RWY samples, resulting in a lower lightness value. Furthermore, the darker yogurt could be due to walnut browning (the Maillard reaction and Caramelization reaction) after roasting.

The CLSM images of walnut yogurt were shown in (Fig. 3(d)). Samples were stained with Nile blue and Nile red to visualize protein colloidal particles and fat globules, respectively. During the fermentation process, the microstructure of the emulsion changes as the pH decreases, culminating in the formation of a gel structure based on protein micelles. Compared with the sample after homogenization, the protein micelles of the non-homogenized group were larger and more unevenly distributed, and the interaction of the protein with the fat globules was not significant (Fig. 3(d1)). The homogenized yogurt samples showed a significant reduction in the size of both fat and protein gel particles and a more prominent contact between them. These reduced-size fat globules were squeezed into and linked to each other under HPH to form a more complete composition (Fig. 3(d2-6)). The independent presence of fat globules and proteins was not observed in the N150 sample (Fig. 3(d6)), which could explain why the yogurt gel structure was stronger after homogenization.

For the RWY group, significantly more fat globules were visible than in the NRWY samples (Fig. 3(d7-9)), indicating that the interaction between fat globules and proteins was not strong and did not form more complete gel particles. Therefore, although the average particle size of the RWY group was smaller than that of the corresponding NRWY



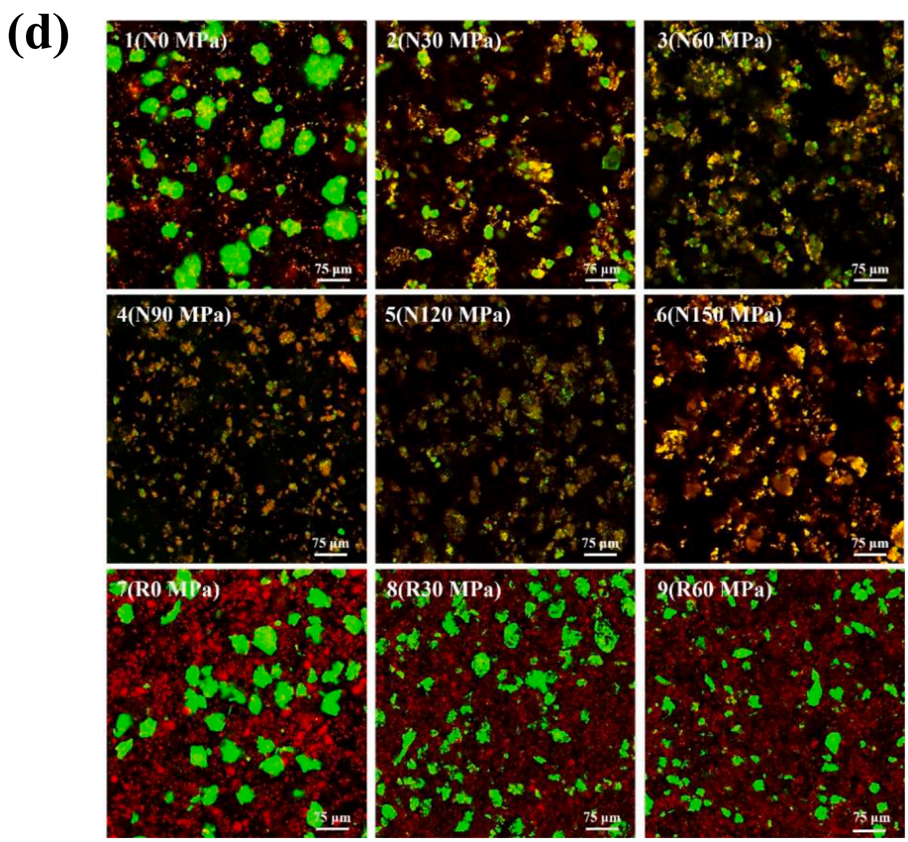


Fig. 3. (*continued*).

samples, their gel strength was inferior to that of yogurt made from the non-roasting walnut feedstock.

3.4. Effect of roasting and HPH on texture and WHC of walnut yogurt

Hardness was considered to be the ability of the specimen to resist deformation before external forces were applied, whereas constancy was the strength of the internal bonding that makes up the bulk of the product. The results showed that hardness and consistency follow the same trend, increasing as the homogeneous pressure increased, and the hardness values of samples treated at 150 MPa were significantly higher than those of other groups ($p < 0.05$) (Fig. 4(a) and (b)). When the raw milk was homogenized, the size of the fat globules and protein gel particles corresponding to the yogurt was significantly reduced. These smaller fat globules were squeezed into the protein space structure by homogenizing pressure and were encapsulated by the protein backbone in the form of fillers, forming tighter aggregates. It might be the main reason for the stronger gel structure of yogurt made with homogenized raw milk, which was macroscopically manifested by a harder, stickier, and more water-holding yogurt. In addition, yogurt produced from raw materials after roasting treatment (RWY group) showed poor hardness and consistency. The differences between these textural properties might be related to the differences in the microstructure of yogurt gels under different treatments and need further exploration.

Water holding capacity was closely related to the microstructure of yogurt and was one of the important indicators for the quality of yogurt, which played a key role in prolonging the shelf life of yogurt products. It was clear that better water-holding properties could be obtained in non-roasting walnut yogurt with homogenous pressure above 120 MPa (Fig. 4(c)). HPH accelerated the unfolding of protein structure and the exposure of hydrophobic groups, facilitated the interaction between *Lactobacillus* and proteins, accelerated the formation of macromolecular aggregates in gels, and improved the gelation properties of the walnut protein. Furthermore, it prevented fat separation during the fermentation process and storage, decreased whey separation, improved consistency, enhanced mouthfeel, and strengthened the gel network (Trujillo et al., 2016).

4. Conclusion

In this study, walnut was found to have potential as a raw material for plant-based yogurt. When walnut milk was treated with HPH above 120 MPa, due to the reduction in particle size and the enhanced interaction between protein and oil, the fermented yogurt had a better gel structure. The resulting walnut yogurt showed better properties in terms of apparent viscosity, hardness, brightness, and WHC. CLSM images of homogenized yogurt samples showed a significant reduction in the size of both fat and protein gel particles, with more prominent contact

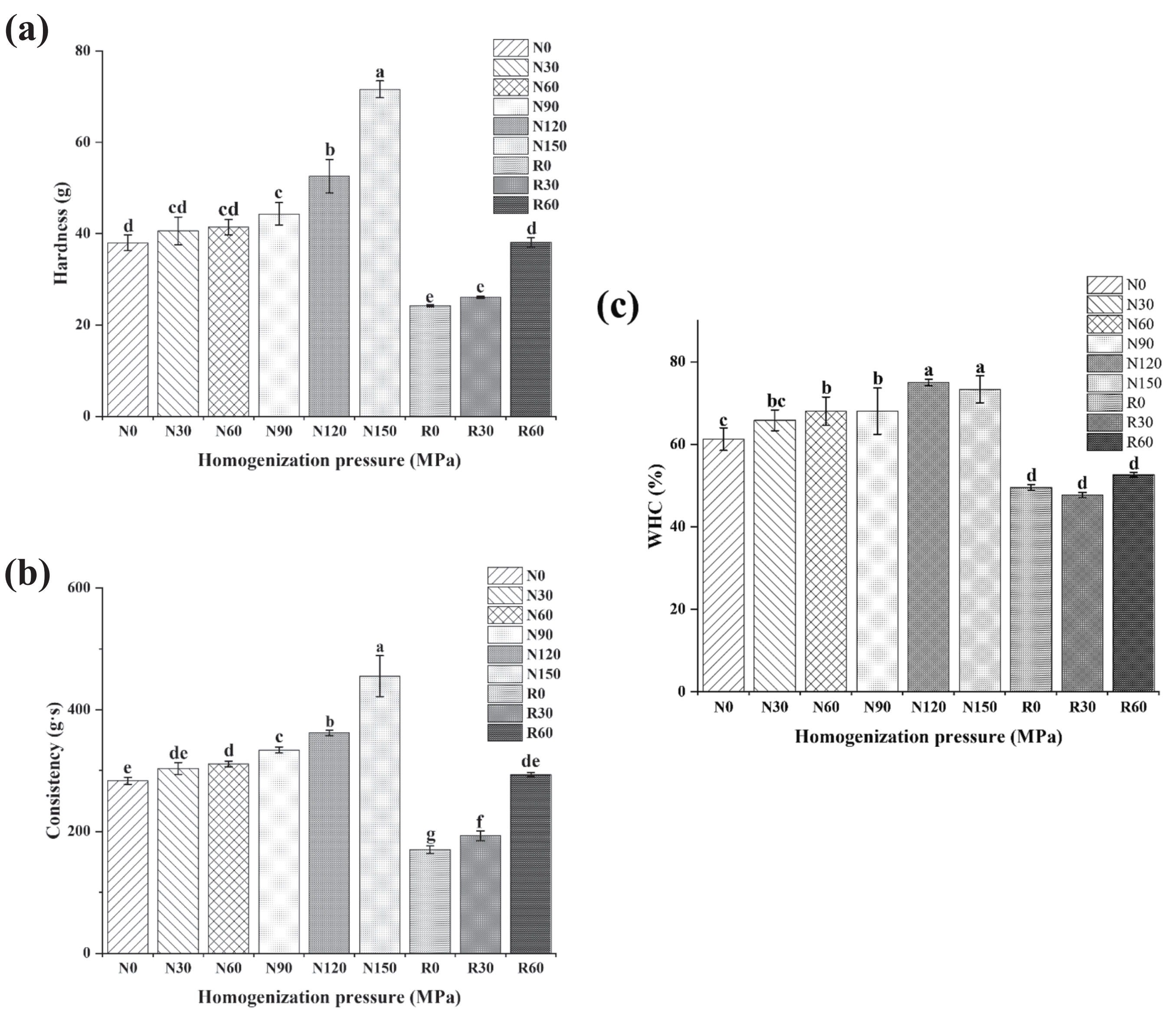


Fig. 4. Changes in textural properties and WHC of yogurt made from roasting (R) and non-roasting (N) walnut under different homogenization pressures (0-150 MPa). (a) Hardness; (b) Consistency; (c) WHC. Values with different letters were significantly different ($p < 0.05$).

between them. These reduced-size fat globules were squeezed and interlinked under HPH to form a more complete composition. In addition, roasted walnut yogurt exhibited shear thinning and lower viscoelasticity. They both showed a reduction in apparent viscosity and yield stress, and the gel structure of the entire yogurt was looser. More research is required to determine the high-temperature roasting parameters appropriate for processing fermented walnut milk.

CRediT authorship contribution statement

Bo Jiao: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Bicong Wu:** Data curation, Writing – original draft, Writing – review & editing. **Weiming Fu:** Data curation, Formal analysis, Investigation, Writing – original draft. **Xin Guo:** Writing – review & editing. **Yu Zhang:** Supervision, Funding acquisition. **Jie Yang:** Supervision. **Xiaohong Luo:** Supervision. **Lei Dai:** Supervision. **Qiang Wang:** Supervision, Funding acquisition, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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