Effect of functional lipids on the quality of walnut butter prepared from defatted walnut meal by ball mill grinding

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Abstract

Abstract In this study, walnut butter was produced by mixing functinal lipids with defatted walnut meal. Three kinds of functional lipids(FL), medium-chain triglycerides (MCT), diacylglyceroe (DG), and conjugated linoleic acid glycerides (CLA), were used to make functional lipids walnut butter(FLWB) and their physical properties as well as microscopic morphology were compared with commercial walnut butters. The FLWBs were prepared by grinding FL and defatted walnut meal through the ball milling technique. The mixing ratios of FL and defatted walnut meal were 6:4, 6.5:3.5, 7:3. It was found that the fluidity of FLWB was increased with the addition amount of FL, but the particle size of FLWB was decreased with the addition amount of DG or CLA. As the additions of DG and CLA increased from 60% to 70%, the D (4,3) of DG-WB and CLA-WB decreased by 36.23% and 20.88%; the flowability index increased by 15.74% and 168.91%. As the addition of MCT increased from 60% to 70%, there was no significant difference in D (4,3) of MCT-WB, but the flowability index increased by 717.34%. Microrheological properties indicated that both FLWB and commercial walnut butter exhibited viscoelastic characteristics. The rheology showed that the FLWB was non-Newtonian pseudoplastic fluids, which was similar to the commercial walnut butter. The DG-WB and CLA-WB were closer to the commercial walnut butter, compared with MCT walnut butter. The microstructure further indicated that the walnut butter with 65% CLA addition was closer to the commercial walnut butter.

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Abstract

In this study, walnut butter was produced by mixing functinal lipids with defatted walnut meal. Three kinds of functional lipids(FL), medium-chain triglycerides (MCT), diacylglyceroe (DG), and conjugated linoleic acid glycerides (CLA), were used to make functional lipids walnut butter(FLWB) and their physical properties as well as microscopic morphology were compared with commercial walnut butters. The FLWBs were prepared by grinding FL and defatted walnut meal through the ball milling technique. The mixing ratios of FL and defatted walnut meal were 6:4, 6.5:3.5, 7:3. It was found that the fluidity of FLWB was increased with the addition amount of FL, but the particle size of FLWB was decreased with the addition amount of DG or CLA. As the additions of DG and CLA increased from 60% to 70%, the D (4,3) of DG-WB and CLA-WB decreased by 36.23% and 20.88%; the flowability index increased by 15.74% and 168.91%. As the addition of MCT increased from 60% to 70%, there was no significant difference in D (4,3) of MCT-WB, but the flowability index increased by 717.34%. Microrheological properties indicated that both FLWB and commercial walnut butter exhibited viscoelastic characteristics. The rheology showed that the FLWB was non-Newtonian pseudoplastic fluids, which was similar to the commercial walnut butter. The DG-WB and CLA-WB were closer to the commercial walnut butter, compared with MCT walnut butter. The microstructure further indicated that the walnut butter with 65% CLA addition was closer to the commercial walnut butter.

Keywords : Defatted walnut meal; Walnut butter; Functional lipids; Vibratory ball mill; Processing characteristics

Introduction

Walnut is one of the most widely grown nut food in the world, which is popular due to its high economic value. China accounts for 48% of the world's walnut productions (Qu et al., 2016). Most walnut is used for walnut oil (WO) extraction, while many defatted walnut meals are produced during this processing. Defatted walnut meal contain more than 40% protein and have high levels of arginine and glutamic acid, while other essential amino acids for the human body (Sun et al., 2019). Defatted walnut meal are often used as animal feed or directly wasted in landfills (Feng et al., 2021). At present, there have been studies on the extraction of active ingredients from defatted walnut meal, such as walnut protein or active peptide (Feng et al., 2021). In addition to this utilization, there is still a need to further improve the comprehensive utilization of defatted walnut meal.

Traditionally, walnut kernels were roasted and then ground to make walnut butter. (Zhaohua et al., 2020). However, the fat content of walnut butter is high, while excessive intake of high-fat foods can increase the risk of obesity and cardiovascular disease (Lu et al., 2019). Therefore, the development of Low-fat and functional walnut butter is becoming a new trend in the nut industry. In order to reduce oil amount of walnut butter, some previous studies produced the product by adding water rather than oil. However, the higher the moisture content of the walnut butter, the more likely it is to have a negative impact on the sensory properties (Mehdi et al., 2019). The oils in walnut butter are beneficial to enhance the processing characteristics and stability of the product, so it is better to use oil as the mobile phase of walnut butter. Therefore, using choice of functional lipids (FL) to replace the oil in traditional nut butters may maintain the quality of nut butter.

FL, such as medium-chain triglycerides (MCT), diacylglyceroe (DG), and conjugated linoleic acid glycerides (CLA), could meet people's need for flavorful oils and reduce fat energy intake. Moreover, FL has many beneficial physiological effects such as losing weight and reducing blood lipid. MCT has good antioxidant stability, which is naturally found in foods such as palm oil and coconut oil (Walker et al., 2017). DG is a trace component of natural vegetable oils, which can be used as a substitute for animal fat in meat processing. These meat products usually had better stability (Miklos et al., 2011). CLA is a physiological active material, which have potentially antitumor and anti-atherosclerosis effects (Belury, 2002).

Traditionally, walnut butter were usually ground by stone mills, high-speed grinders, and colloid mills (Wagener and Kerr, 2018). It is hard to obtain small walnut butter particle sizes by one time of grinding using the above mentioned traditional grinding methods. So a secondary grinding is necessary for walnut butter to reach a specific particle size range. Vibration ball milling is a new ultrafine grinding technology. Ball milling could exert collisional, compressive, shear, and frictional forces on the sample during the collision processing. The frictional process could lead to local heating, causing thermochemical reactions (Steiner et al., 2016). Therefore, after ball milling treatment, the processed material undergoes reversible internal structural rearrangement. This process can create new active surfaces or result in covalent bond breakage, and thus changing the molecular conformation and chemical bonding of the material (Karinkanta, 2014; Marti, 2004). Based on the above mentioned information, ball milling technology is gaining more and more attention as a new, efficient and green grinding technology in the food field. Vibration ball milling may effectively reduced the particle size of walnut butter with only one time of grinding. Consequently, this study aimed to make comprehensive utilization of defatted walnut meal to make a healthier and marketable FLWB by vibration ball milling technology.

Materials and Methods

2.1 Materials

Commercial walnut butter-1 (SS1, purchased from Shengyuan Agricultural Technology Co., Ltd), commercial walnut butter-2 (SS2, purchased from Tianjin Jizhou Green Food Group Co., Ltd), commercial walnut butter-3 (SS3, Purchased from Nanshizhao Food Group Co., Ltd); defatted walnut meal(purchased from Hebei Chenxing Biotechnology Co., Ltd); medium-chain triglyceride(purchased from Wuhan Kangcan Biotechnology Co., Ltd), diglyceride(purchased from Xi'an Huageng Biotechnology Co., Ltd), and conjugated linoleic acid lipid (Purchased from Qingdao Aohai Biotechnology Co., Ltd, all three FL are food grade). All chemicals were purchased from Sinopharm Chemical Reagent Co.,Ltd. They included: Methyl red indicator, methyl blue indicator, petroleum ether, sodium dodecyl sulfate.

2.2 Preparation of FLWB

Preparation of FLWB was according to a previous method with optimization (Mostafa et al., 2019). The oil phase (MCT, DG, CLA) and defatted walnut meal were premixed in the weight ratios of 6:3, 6.5:3.5 and 7:3. Then the premixed samples were further ground in a ball milling (RETSCHMM 400, Retsch, Germany) tank. The walnut butter prepared with three functional lipids were noted as MCT-WB, DG-WB, CLA-WB. In the case of MCT: walnut butter prepared with 60%, 65% and 70% MCT was noted as MCT-6, MCT-6.5 and MCT-7. The FLWB was stored in 25 mL sealed sample bottles at 4 ± 2°C (taken within three days). Before analysis, the samples were removed from the freezer and kept at 25 ± 2°C for 1 h for subsequent tests.

2. 3 Measurement of particle size

Particle size measurement are performed according to the previous method (Liu et al., 2007). The walnut butter was dissolved in sodium dodecyl sulfate (SDS) solution (0.1%) at a ratio of 1:50 (w/v), then stirred with a magnetic stirrer and shook with a vortex shaker until the walnut butter was well dispersed. The particle size of the samples was measured using a particle size analyzer (Mastersizer 2000, Malvern, UK). The walnut butter to be measured was added to 1000 mL of deionized water and the stirring paddle speed was set to 2000 rpm. The degree of shading was kept in the range of 10 - 15%. The refractive index of the dispersed phase (aqueous phase) was 1.330. The refractive index of the oil phases is shown in Table S1. The volumetric mean particle size D (4, 3) was used to indicate the sample particle size. The measurements were repeated at least three times for each sample.

2. 4 Color of walnut butter

The determination of color was referenced to a previous method (Sun et al., 2015). The color parameters of walnut butter were determined with a colorimeter (Ultrascan VIS, HuntereLab, USA). Where L* reflected the brightness of the product, a* indicated a red-green value and b* indicated a yellow-blue value. The measurements were repeated at least three times for each sample.

2.5 Determination of rheological properties

2.5.1 Measurement of oil viscosity

The rheological properties of walnut butter were measured based on a previous method (Mostafa et al., 2019). The viscosity of the oil was determined using a DHR 2 rheometer (Waters, USA). Experimental conditions: an aluminum plate of 60 mm with a taper of 1.007° was selected, plate spacing was set to 500 μm, the temperature was set to 25°C, and the frequency variation range was set to 1 - 100 s^{-1}.

2.5.2 Measurement of apparent viscosity

The apparent viscosity of walnut butter was measured with the shear rate from 1 s^{-1} to 100 s^{-1} using a rheometer at 25°C. The hydrodynamic variation curves of the walnut butter were recorded.

2.5.3 Frequency scanning

Strain scan results were used to determine the linear viscoelastic region (LVE) of the walnut butter. The samples were placed as uniformly as possible between the plates. The frequency was fixed at 10 rad/s or 1 Hz and the stress variation range was set to 0.01% - 100%.

The viscoelastic properties of the walnut butter was determined by selecting the small amplitude dynamic frequency scan mode. Depending on the measurement, the strain was fixed at 0.01% (linear viscoelastic region). In the linear state, the scanning frequency was set from 0.1 rad/s to 100 rad/s. The numerical changes of the energy storage modulus (G') and the energy dissipation modulus (G") were recorded.

2.5.4 Determination of thixotropy

A two-step steady state flow procedure was used to increase the shear rate from 0 s^{-1} to 150 s^{-1}. And immediately afterward decrease it from 150 s^{-1} to 0 s^{-1} at the same rate of change. The variation of shear stress and viscosity with the shear rate were recorded throughout the process. The obtained data were simulated curvilinearly using the cross model.

2.6 Microrheological characterization

The microrheological properties were determined with reference to a previous method (Mengjie et al., 2021). The microrheological properties of walnut butter were investigated using a microrheometer (Rheolaser Master, France). The freshly prepared walnut butter was placed in a 4 mL cylindrical glass tube to observe the Brownian Motion of the particles. The setting time was 4 h, and the temperature was 25°C. The changes in the mean square displacement (MSD) curve, the elasticity index (EI), solid-liquid balance index (SLB), and the flow index (FI) of the sample particles were recorded using Rheosoft Master software 1.4.0.0 software. The elasticity index (EI), solid-liquid balance index (SLB), and the flow index (FI) were obtained from the mean square displacement (MSD) curves. EI was the inverse of the height value of the plateau zone in the MSD curve. SLB was the slope value of the elastic plateau zone of the MSD curve. FI was the inverse of the characteristic decorrelation time corresponding to the decorrelation time required to reach the correlated scatter plot (Fernandes and Salas Mellado, 2018).

2.7 Microstructure of walnut butter

The microstructures were observed using a Nikon Eclipse Ti-S (Nikon Instruments Inc, USA) microscope. After taking 1 μL of sample on a slide and smearing it well, the microstructure of FLWB was observed with a 40x objective. The images were acquired as quickly as possible for better image assurance.

3. Results and discussion

3.1 Particle Size Distribution

As shown in Fig. 1, the particle size distribution curves of the three commercially available walnut butter were similar (Fig. 1A). However, from Fig. 1 B it was observed that SS3 had the smallest D (4,3) value (33.55 $\pm$ 0.41 μm), which was significantly different from SS1 (50.76μm) and SS2 (55.14μm) ($P<0.05$). The fluidity of walnut butter increased with the reduction of particle size, which may result in a finer texture when consumed (Nam et al., 2014). Compared to commercial walnut butter, the FLWB prepared with MCT, DG, or CLA and defatted walnut meal showed a single-peak particle size distribution with a central peak width range of 1.78 – 796.21 μm for all samples (Fig. 1A). It was noticed that adding 65% of CLA or 70% CLA significantly moved the prominent peak of the particle size distribution to the left. It was found that the average particle size of DG walnut butter and GLA walnut butter decreased with increasing oil addition ratio. However, the average particle size of MCT walnut butter showed no significant difference at different MCT addition ratios. As the addition of DG or CLA increased from 60% to 70%, the average particle size of walnut butter decreased by 36.23% and 20.88%, respectively (Fig. 1B). The particle size of commercial walnut butter varied considerably as shown in D (4,3) for SS1, SS2, and SS3. The particle size of MCT-WB did not vary with the addition of MCT, but the particle size of DG-WB or CLA-WB could be adjusted by adjusting the addition of DG or CLA, which was used to prepare a product with a particle size more similar to that of commercial walnut butter. The above results indicated that the particle size data of FLWB could have similar characters with commercially walnut butter after ball milling treatments.

3.2 Color of walnut butter

Color was usually an unappreciated physical parameter (Jose Moyano et al., 2010). However, the study by Gambaro showed that color could influence the preferences of consumers (Gambaro et al., 2014). In the color analysis, L* represented lightness and darkness (black and white), a* represented red-green, and b* represented yellow-blue. Table 1 represented the color difference results of different walnut butter. There were significant differences ($p<0.05$) in the L*, a*, and b* between the FLWB and commercial walnut butter. MCT-WB had slightly higher L* than SS1 and SS2. The L*, a* and b* of DG-WB and CLA-WB were lower than those of commercial walnut butter. As a result, the color of the sauces showed different degrees of yellow color for commercial walnut butter and FLWB. The b* of FLWB was significantly lower than the commercial walnut butter. The oil in traditional walnut butter was mainly derived from the walnut oil in the walnut kernels, and its color was mainly influenced by the beta-carotene contained in the walnut oil (Misawa, 2009). The carotenoid content in walnuts ranged from 0.08-0.49 mg/kg (Ozrenk et al., 2012), which was resulting in a higher yellow value of walnut oil (Martínez et al., 2008). As a result, walnut oil had a more yellow color due to its highest carotenoid content, while other oils appear lighter yellow or even colorless. Walnuts could lose the carotenoids after the extraction of walnut oil, which caused that the defatted walnut meal lost the original color of walnuts and took on a grayish-yellow color. This could also affect the color of walnut butter which could appeared light-yellow or grayish-yellow. It may also be the reason why commercial walnut butter containing walnut oil is more yellowish than walnut butter prepared with FL. Tables 2S also showed differences in the color of the four oils. There is a correlation between the prepared walnut butter and FL, which may account for the differences in the color of the walnut butter.

3.3 Microrheological characterization

3.3.1 Apparent viscosity of walnut butter

During the ball milling process, MCT, DG and CLA formed different emulsification structures with the defatted walnut powder, which resulted in different textures of walnut butter. As the viscosity of the oil increased, the stability and sensory acceptability of walnut butter was improved. However, when the viscosity of oil beyond a certain range, it could have a negative impact on the spreadability of low-fat walnut butter (Fernandes and Salas Mellado, 2018). The viscosity of WO, MCT, DG, and CLA does not vary with shear rate (Fig. 2A). The viscous of CLA was the largest and was closer to walnut oil, while MCT and DG were less viscous.

The rheological properties of walnut oil play a crucial role in food processing. Fig. 2 showed the results of the apparent viscosity measurements of commercial walnut butter and functional lipids walnut butter at a temperature of 25°C. The results showed that the apparent viscosity of all samples showed a gradual decrease with increasing shear rate at the same temperature. This phenomenon indicated that walnut oil has non-Newtonian shear thinning properties (Mostafa et al., 2019). Similar phenomena have been found in sesame butter, peanut butter and mayonnaise (Loncarevic et al., 2016; Muresan et al., 2014; Yang et al., 2020). The presence of high molecular weight substances and aggregation of polymers, such as xanthan gum, walnut proteins and polysaccharide bonds, were also responsible for their high shear thinning behavior (Vardhanabhuti and Ikeda, 2006). In addition, with the increase of external shear force, the agglomerated particles in the emultion system could continue to deform and break. This could reduce the flow resistance of lotion, which also resulted in the reduction of its apparent viscosity (Mun et al., 2009). The apparent viscosity of MCT-6.5 was significantly higher than that of MCT-6 and MCT-7 (Fig. 2D). It may be due to the better stability of the formed emulsion agglomerate structure at the ratio of oil: defatted walnut meal was 6.5:3.5, exhibiting a higher apparent viscosity at low shear rates. The apparent viscosity of DG-WB or CLA-WB decreased with the increase of oil addition ratio (Fig. 2B and 2C). It may be due to that more oil acted as the mobile phase of the system, which resulting in a more effective arrangement of small particles in the flow direction and a lower degree of intermolecular entanglement (Marti, 2004). Fig. 2 showed that, the apparent viscosities of CLA-6.5, CLA-7, DG-6.5 and MCT-6.5 are closer to those of commercial walnut butter.

3.3.2 Frequency scan of walnut butter

The linear viscoelastic region (LVE) was the region where the elastic modulus (G') and viscous modulus (G") of all samples did not vary with the oscillatory stress under the same conditions (Bi et al. 2020). The LVE of walnut butter could be determined by strain scanning in the dynamic rheological oscillation measurement mode. To ensure that all samples were in the linear viscoelastic region, a strain value of 0.01% was selected for subsequent experiments (Fig. 1S).

Frequency scanning is a standard method for studying the viscoelastic behavior of substances, which helped study the chemical composition and physical structure of substances (Daubert, 2017; Steffe, 1996). The results showed that the G' values of commercial walnut butter and three groups of FLWB were always more significant than the G" values in the frequency range of 0.1-100 Hz (Fig. 3). There was no crossover between G' and G", which indicating that commercial walnut butter and FLWB were dominated by elastic properties. There was a weak frequency dependence of G' and G" of the samples, suggesting that aggregation and cross-linking between the components of walnut butter occurred. The G' and G" values of walnut butter decreased with the increase of FL addition, indicating that the particle size of the samples decreased with the increase of oil addition (Fig. 3B - D). The results showed the trend of walnut butter from elasticity to viscosity was increased, the distance between oil droplets was decreased, and the flocculating trend appeared between microdroplets. This led to enhanced fluid interaction and increased viscosity (Barnes, 1994; Sato et al., 2015). In all walnut butter samples, the G' values were higher than the G" values in all frequency ranges. The G' values indicated strong interparticle interactions and stable network structures within the samples, and the same phenomenon was observed in sesame butter (Çiftçi et al., 2008).

3.3.3 Thixotropy of walnut butter

Thixotropy could reflect the phenomenon that the structure was disrupted and the viscosity of the system decreases when shear was applied to a system. After the removal of shear, the sample could gradually and reversibly return to the initial stress and viscosity, which was typical for non-Newtonian fluids (Hadjistamov, 2019). The thixotropic behavior was related to the "untangling-entanglement" process of protein molecules and the alignment of molecular protein chains in the shear direction (Benchabane and Bekkour, 2008).

When the shear rate applied to commercially available walnut butter SS1, SS2 and SS3 increased and then decreased, the viscosity curves of walnut butter did not overlap while formed a closed loop region surrounded by the upper and lower curves of viscosity, which could be called thixotropic loop (Fig. 4A). The thixotropic ring area was negatively correlated with the reversibility of viscosity, while the sample recovered gradually when the shear rate was reduced (Ashok et al., 2015). Firstly, the formation of the thixotropic ring could be due to the non-covalent interaction between proteins in walnut butter at the initial stage of shearing, which formed a three-dimensional network structure with more intermolecular entanglement points and higher shearing resistance. A linear increase in the applied shear rate during the shearing process resulted in a gradual deformation between the walnut butter particles and a disruption of the three-dimensional network structure. This increased the number of broken structures and decreasing the viscosity (Nikzade et al., 2012).

During the shearing process, the linear increase of applied shear rate led to gradual deformation between walnut butter droplets. As a result, the protein three-dimensional network structure was disrupted and the viscosity of walnut butter was reduced (Reza et al., 2019). Secondly, the thixotropy size was related to the intermolecular forces. Some researches about the thixotropy of guar gum and papaya seed gum found that the stronger the intermolecular interact ions, the greater the structural strength and the longer the time required for structural rearrangement (Wang et al., 2019). DG-7, CLA-6 and CLA-7 formed distinct thixotropic loops (Fig. 4B - D). In addition to this, the initial viscosity values of CLA-6.5 and CLA-7 were closer to those of commercial walnut butter. In summary, the thixotropy of CLA-WB was more similar to that of commercial walnut butter.

3.4 Microrheological characterization

Microrheological techniques could monitor the Brownian Motion of tracer particles and track interparticle interactions (Mengjie et al., 2021). The Brownian motion of the particles is described by the MSD versus time curve, which reflects the viscoelastic characteristics of the sample. The interaction forces between the model particles with viscoelasticity limit their mean square displacement. Since the particles are not free to move, they are trapped in a three-dimensional microstructural network. These interactions make the samples viscoelastic, which is manifested in the root mean square displacement versus de-correlation time curves as non-linear MSD for all samples. Fig. 5 shows the results for the root mean square displacement of walnut butter particles. The MSD curves of commercial walnut butter and all three groups of FLWB were nonlinear. It indicates that both commercial walnut butter and FLWB are typical viscoelastic products (Fernandes and Salas Mellado, 2018; Tisserand et al., 2012). MCT-WB and DG-WB had a long decorrelation time, while commercial and CLA-WB had a shorter decorrelation time. The samples with longer decorrelation times had higher viscoelasticity when moved the same distance (Yun et al., 2018).

Fig. 6 showed the results of elasticity index (EI), solid-liquid balance (SLB) and fluidity index (FI) of walnut butter. the EI value of FLWB tended to decrease with increasing amount of grease addition and the fluidity of walnut butter was enhanced, which was consistent with the results of frequency scan in rheological experiments. the FI value of FLWB increased with increasing amount of oil addition. This indicates that the particle migration rate and fluidity of FLWB were enhanced with the addition of oil. The FI of DG-WB was higher than that of commercial walnut butter, and this phenomenon may be due to the relatively low viscosity of DG, resulting in the higher fluidity of DG-WB. With the increase of oil addition, the FI of MCT-WB or CLA-WB gradually approached the FI of commercial walnut butter (Fig. 6C). Therefore, it was feasible to prepare samples of MCT-WB or CLA-WB by adjusting the amount of oil addition to make them closer to commercial walnut butter. Fig. 6B showed that SLB < 0.5 for all walnut butter except for the MCT-7 sample (which was mainly liquid behavior), indicating that the samples were mainly gel behavior. In summary, the microscopic rheological properties of CLA-WB are closer to those of commercial walnut butter.

3.5 Microstructure analysis

In conclusion, among FLWB, CLA-WB were closer to the quality of commercial walnut butter. Therefore, the walnut butter with CLA addition was selected for further microstructural analysis. Image analysis provided information on the pixel organization of the different regions of the samples (Backes and Bruno, 2013). The walnut butter samples prepared with CLA were further analyzed using optical microscopy Fig. 7 showed the optical microstructure of walnut butter prepared by CLA and commercially available walnut butter. The results indicated that all walnut butter showed a porous matrix structure encasing the oil droplets. Proteins were adsorbed on the surface of the oil droplets while some of them formed aggregates between the oil droplets and the proteins. The aggregation of SS1 and SS2 was higher while the oil droplet size of SS1 was more homogeneous. Emulsion droplets with smaller average particle size may result in a larger total interfacial area of emulsion droplets and more adsorbed proteins (Farshchi et al., 2013), thus preventing aggregation between oil droplets. The formation of high oil content and low interfacial adsorbed proteins may result in unstable oil droplets that tended to form aggregated large oil droplets (Fig. 7F). Due to protein and oil droplet interactions, FLWB had a particle size and emulsion structure, which was similar to that of commercial products (Xu et al., 2020). The results showed that the quality of CLA-WB was closer to that of commercial walnut butter. Further microscopic results showed that the particle size, distribution and aggregation of CLA-6.5 were closer to the microstructure of commercial walnut butter.

Conclusion

In this study, different types and additions of FL (MCT, DG, CLA) were selected to prepare walnut butter. By measuring the physicochemical properties of the three groups of samples, it was obtained that when the addition ratio of CLA and defatted walnut meal was 6.5:3.5, the prepared walnut butter had the similar properties to commercial walnut butter. FLWB with similar processing physical properties and microscopic morphology as commercial walnut butter could be obtained after vibratory ball mill grinding. In addition, FLWB contained 65% CLA had higher bioactive functions. This study provided a new way for the comprehensive development and utilization of defatted walnut meal, which could help the development of functional lipids walnut butter. Vibratory ball milling technology was used to grind walnut butter in a single process to streamline the milling process. which provided a new method for the preparation of functional lipids walnut butter.

Conflict of Interest Statement

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.

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Authorship:

Y.X. Conceived and designed the study, and wrote the first draft of the manuscript, N.J. Carried out the research, H.S. and F.T. Analyzed the data, X.C. and J.W. Writing – review & editing. All authors contributed to and approved the final draft of the manuscript.

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Fig. 1 Particle size distribution of commercial walnut butter and walnut butter prepared with MCT, DG and CLA(A).Effect of functional fats on walnut butter D(4,3) (B): [SS1, SS2, SS3 (Three types of commercial walnut butter); Amount of oil added: MCT-6(60%MCT), MCT-6.5(65%MCT), MCT-7(70%MCT); DG-6(60%DG), DG-6.5(65%DG), DG-7(70%DG); CLA-6(60%CLA), CLA-6.5(65%CLA), CLA-7(70%CLA)]

Fig. 2 Apparent viscosity of walnut oil, MCT, DG and CLA(A).Apparent viscosity of walnut butter prepared by CLA、DG、MCT(B、C、D); Apparent viscosity of commercial walnut butter(E): [SS1, SS2, SS3 (Three types of commercial walnut butter); Amount of oil added: MCT-6(60%MCT), MCT-6.5(65%MCT), MCT-7(70%MCT); DG-6(60%DG), DG-6.5(65%DG), DG-7(70%DG); CLA-6(60%CLA), CLA-6.5(65%CLA), CLA-7(70%CLA)]

Fig. 3 Frequency scan of commercially available walnut butter(A) Frequency scanning of walnut butter prepared by MCT, DG and CLA(B、C、D): [SS1, SS2, SS3 (Three types of commercial walnut butter); Amount of oil added: MCT-6 (60%MCT), MCT-6.5 (65%MCT), MCT-7 (70%MCT); DG-6 (60%DG), DG-6.5 (65%DG), DG-7 (70%DG); CLA-6 (60%CLA), CLA-6.5 (65%CLA), CLA-7 (70%CLA)]

Fig. 4 Thixotropy of commercially available walnut butter(A) Thixotropy of walnut butter prepared by MCT, DG and CLA(B、C、D): [SS1, SS2, SS3 (Three types of commercial walnut butter); Amount of oil added: MCT-6 (60%MCT), MCT-6.5 (65%MCT), MCT-7 (70%MCT); DG-6 (60%DG), DG-6.5 (65%DG), DG-7 (70%DG); CLA-6 (60%CLA), CLA-6.5 (65%CLA), CLA-7 (70%CLA)]

Fig. 5 MSD curve of walnut butter: [SS1, SS2, SS3 (Three types of commercial walnut butter); Amount of oil added: MCT-6 (60%MCT), MCT-6.5 (65%MCT), MCT-7 (70%MCT); DG-6 (60%DG), DG-6.5 (65%DG), DG-7 (70%DG); CLA-6 (60%CLA), CLA-6.5 (65%CLA), CLA-7 (70%CLA)]

Fig. 6 Texture characteristics of walnut butter ($p<0.05$): [SS1, SS2, SS3 (Three types of commercial walnut butter); Amount of oil added: MCT-6 (60%MCT), MCT-6.5 (65%MCT), MCT-7 (70%MCT); DG-6 (60%DG), DG-6.5 (65%DG), DG-7 (70%DG); CLA-6 (60%CLA), CLA-6.5 (65%CLA), CLA-7 (70%CLA)]

Fig. 7 Microstructure of walnut butter prepared by CLA compared with commercial walnut

butter [SS1, SS2, SS3 (Three types of commercial walnut butter); Amount of oil added: CLA-6 (60%CLA), CLA-6.5 (65%CLA), CLA-7 (70%CLA)]

Table 1 Marketable and homemade walnut butter color difference results

Walnut butter types	L*	a*	b*
SS1	57.19 ± 0.30^{c}	3.97 ± 0.07^{ab}	22.91 ± 0.21^{a}
SS2	57.31 ± 0.19^{c}	4.00 ± 0.20^{a}	23.38 ± 0.77^{a}
SS3	59.43 ± 0.62^{a}	3.34 ± 0.15^{d}	22.80 ± 0.52^{a}
MCT-6	57.60 ± 0.60^{bc}	3.15 ± 0.17^{de}	16.70 ± 0.37^{ef}
MCT-6.5	58.21 ± 0.40^{b}	3.15 ± 0.14^{de}	16.39 ± 0.24^{f}
MCT-7	58.23 ± 0.49^{b}	3.02 ± 0.09^{e}	16.17 ± 0.47^{f}
DG-6	52.91 ± 0.17^{d}	3.71 ± 0.06^{c}	18.77 ± 0.44^{b}
DG-6.5	52.94 ± 0.58^{d}	3.61 ± 0.14^{c}	18.40 ± 0.50^{bc}
DG-7	52.54 ± 0.34^{d}	3.60 ± 0.14^{c}	18.12 ± 0.81^{c}
CLA-6	46.74 ± 0.22^{e}	3.94 ± 0.09^{ab}	17.42 ± 0.29^{d}
CLA-6.5	45.10 ± 0.60^{g}	3.77 ± 0.06^{bc}	16.82 ± 0.22^{def}
CLA-7	45.98 ± 0.45^{f}	3.56 ± 0.20^{c}	17.24 ± 0.12^{de}

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