Fabrication and characterization of Pickering high internal phase emulsions stabilized by Tartary buckwheat bran flour

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ABSTRACT

The abilities of Tartary buckwheat whole flour (TBWF), Tartary buckwheat core flour (TBCF), and Tartary buckwheat bran flour (TBBF) to directly construct Pickering high internal phase emulsions (HIPEs) were compared. The results indicated that these flours had a similar appearance and size distribution, but TBBF was rich in proteins, lipids, and rutin. As a result, its hydrophobicity and ability to reduce interfacial tension were superior to TBWF and TBCF. These flours could fabricate HIPEs with an oil phase volume fraction ($\phi$) of 80%, and the increase in the addition amount (c) was not only beneficial to the mechanical properties, but also accelerated gel formation. When c was constant, the HIPEs developed by TBBF exhibited the smallest droplet size and the highest gel strength. The sunflower oil-based HIPE developed by TBBF at $\phi = 80\%$ and $c = 5\%$ could also partially replace butter for cake preparation, and improve antioxidant activity of cakes.

1. Introduction

Plastic fats, such as shortening and margarine, are usually used in food processing, and their main sources are animal fats and hydrogenated vegetable oils (Saghafi, Naeli, Bahmaei, Tabibiazar, & Zargaraan, 2019). The former is rich in saturated fatty acids, while the latter contains a considerable amount of trans fatty acids. Their excessive intake can increase the risk of coronary heart disease, diabetes, and cancer (Pehlivanoglu, Demirci, Toker, Konar, Karasu, & Sagdic, 2018). Therefore, it is imperative to find fat substitutes that are safe and exhibit good processing characteristics.

Pickering emulsion is a new development of emulsion technology with solid particles as emulsifier instead of traditional surfactants, which has the advantages of high stability and easy preparation (Xiao, Li, & Huang, 2016). Pickering high internal phase emulsions (HIPEs) are Pickering emulsions that have a disperse phase volume fraction of $\geq 74\%$ (Liu, Li, Geng, Mo, & Liu, 2021). Vegetable oil-based Pickering HIPEs stabilized by colloidal particles assembled from polysaccharides and proteins have attracted widespread attention as potential fat substitutes, offering health benefits while maintaining suitable processing characteristics (Abdullah et al., 2020). Unfortunately, these food-derived macromolecules have strong hydrophilicity and poor emulsifying capacity, and they must be subjected to pretreatments such as acid hydrolysis and esterification (Sweedman, Hasjim, Schäfer, & Gilbert, 2014), which are time-consuming and environmentally unfriendly. In addition, the safety of the final product needs be confirmed. As a result, the practical applications of Pickering HIPEs are limited. In our previous study, we found that high-amylose corn starch combined with dihydromyricetin could induce the formation of Pickering emulsions using a one-step high-speed shearing process at room temperature, and the content of dihydromyricetin in the complex was positively correlated with the mechanical performance and stability of the emulsions (Geng, Liu, Ma, Liu, & Liang, 2021). This may be due to the fact that flavonoids have a certain Pickering emulsifying ability (Luo, Murray, Ross, & Povey, 2012; Geng, Ma, Pu, Liu, & Liang, 2021), and when they are combined with the colloidal particles of biomacromolecules, they can modify the surface of these particles and improve their emulsifying ability (Geng, Liu, Ma, Liu, & Liang, 2021). Therefore, the flavonoid-rich food materials can be directly used to stabilize Pickering emulsions and endow the final products with a variety of biological functions, such as antioxidant activity.

Tartary buckwheat (Fagopyrum tataricum L.), a dicotyledonous buckwheat crop in the Polygonaceae family, is widely distributed throughout China, Russia, South Korea, Japan, Europe, and other regions (Lothar, Golob, Germ, Vombergar, & Kreft, 2021). Compared to wheat, rice, corn, and other cereals, Tartary buckwheat is rich in flavonoids such as rutin, and has a higher nutritional and medicinal value. Luo et al. found that buckwheat tea extract could stabilize sunflower oil-
based Pickering emulsions when used as the aqueous phase (Luo, Murray, Ross, & Povey, 2012); however, the oil phase volume fraction ($\phi$) of the obtained emulsion was only 20%, which failed to meet the requirement of $\phi \geq 74\%$ for HIPEs. We speculated that this was possibly due to the low rutin content of the aqueous phase. In a previous study, we combined mechanical grinding and sieving to prepare Tartary buckwheat whole flour (TBWF), Tartary buckwheat core flour (TBCF), and Tartary buckwheat bran flour (TBBF), and evaluated their processing characteristics (Zhang, Chen, Geng, Liu, & Gao, 2021). In this study, we first systematically investigated the chemical composition and particle properties of these flours, and compared their abilities to form Pickering HIPEs. Then, we characterized the structure and properties of the obtained HIPEs. Finally, we evaluated the feasibility that the HIPEs developed by TBBF replaced butter for cake preparation.

2. Materials and methods

2.1. Materials and chemicals

Tartary buckwheat (variety, Jinqiao 2) was provided by the Institute of Crop Science, Shaxi Agricultural University (Taiyuan, China), and the pastry flour was produced by COFCO FLOUR Industry Co., Ltd. (Haining, China). Sunflower seed oil was obtained from Yihai Kerry Oils, Grains & Foodstuffs Industries Co., Ltd. (Shanghai, China), and anchor butter was purchased from Fonterra Co-operative Group Ltd. (Taranaki, New Zealand). In addition, the baking powder was produced by Guilin Kesheng Food Co. Ltd. (Guilin, China), and 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteau reagent, rutin, Nile Red, and Nile Blue A were purchased from Sigma-Aldrich Chemical, Inc. (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2. Preparation of TBWF, TBCF, and TBBF

Preparation of TBWF, TBCF, and TBBF was carried out according to the method described in our previous work (Zhang, Geng, Shi, & Liu, 2021). After the Tartary buckwheat was ground using a Chopin CD1 laboratory mill (Paris, France), the hull and TBWF were separated using a 26GG sieve. TBWF was then further screened into TBCF and TBBF using a 7XX sieve (Fig. S1), and the yields of TBWF, TBCF, and TBBF were 73.1%, 48.5%, and 24.6%, respectively. These flours were further crushed by a high-speed universal grinder (Boou Hardware Products Co., Yongkang, China) and sifted through 100 mesh for the following experiments.

2.3. Chemical composition determination

The moisture, mineral, lipid, protein, and starch contents of TBWF, TBCF, and TBBF were measured according to the Chinese national standards GB 5009.3–2016, GB 5009.4–2016, GB 5009.6–2016, GB 5009.5–2016, and GB/T 20194–2018, respectively. The rutin content in the sample was extracted with 80% ethanol and determined by the external standard method using an Agilent 1260 HPLC system (Santa Clara, CA, USA) with an Agilent ZORBAX SB-C18 column (4.6×150 mm, 5 μm particle size) (Zhang, Geng, Shi, & Liu, 2021).

2.4. Particle appearance and size measurement

TBWF, TBCF, and TBBF were evenly distributed on specimen stubs with double-sided adhesive tape and sprayed with gold. Afterward, the samples were observed with an FEI Quanta 200 environmental scanning electron microscope (Hillsboro, OR, USA) at an accelerating potential of 15 kV under low vacuum (Geng, Jiang, Ma, Pu, Liu, & Liang, 2021), and the particle size distributions of TBWF, TBCF, and TBBF were determined using a BETTER BT-9300H laser particle analyzer (Dandong, China).

2.5. Interfacial tension determination

The interfacial tension between the sample dispersion and sunflower oil was investigated using a Theta Lite optical contact angle meter (Biologic Scientific, Sweden) (Gomez-Luria, Vernon-Carter, Alvarez-Ramirez, & Cruz-Sosa, 2019). The sample (0.5 g) was added to 50 mL of distilled water, extracted under ultrasonication at 25 °C for 10 min, and then allowed to rest for 15 min. The supernatant was collected as the sample solution and loaded into a high-precision syringe. Then, to form the oil-water interface, a drop of sample solution was added to a cuvette containing sunflower oil. Then, the interfacial tension was calculated by recording the axisymmetrical drop shape and fitting it with the Young-Laplace equation.

2.6. Contact angle measurement

The water-in-air contact angle ($\theta$) of the sample was determined using a Theta Lite optical contact angle meter (Biologic Scientific, Sweden). The sample was pressed into a film at 20 MPa of pressure and subsequently placed on a platform. A drop of distilled water was then extruded from a high-precision syringe and released onto the film surface. The drop shape was continuously recorded and calculated using the Ellipse fitting model to obtain the contact angle (Wei & Huang, 2019).

2.7. Preparation of the Pickering emulsions

According to the previous report (Geng, Jiang, Ma, Pu, Liu, & Liang, 2021), different amounts of sample (c, 1–5 %) were dispersed in distilled water, which was used as the aqueous phase, while the sunflower oil was used as the oil phase. Then, the oil and aqueous phases were mixed at different oil phase volume fractions ($\phi$, 50–90 % w/w), and homogenized at 12000 rpm for 3 min using an IKA Ultra-Turrax T18 disperser (Staufen, Germany). The resulting mixture was transferred into a 25 mL flat-bottom glass bottle and stored for 24 h at 4 °C for subsequent experimentation. The formation of the emulsion gel was confirmed using the inverted-tube method.

2.8. Optical microscopy

The emulsion (20 μL) developed at $\phi = 80\%$, $c = 1$, 3, 5 % was diluted 2 times with water and deposited onto a glass slide and covered with a coverslip, and morphology observation was carried out using a BH200P polarizing microscope equipped with a digital camera (Shanghai Sunny Hengping Scientific Instrument Co., Ltd., Shanghai, China). Afterward, size distribution analysis of the droplet was performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA) based on the previous report (Xiao, Lo, & Huang, 2015).

2.9. Confocal laser scanning microscopy

According to a published report (Boostani, Hosseini, Golmakani, Marefat, Hadi, & Rayner, 2020), the aqueous and oil phases were pre-stained with 0.1 mg/mL Nile Blue A (in water) and 0.01 wt% Nile Red (in 1,2-propanediol), respectively, and then homogenized. The obtained emulsion was observed at 2000× using a Carl Zeiss LSM780 confocal laser scanning microscope (Oberkochen, Germany), and Nile Red and Nile Blue A were excited at 488 nm and 633 nm, respectively. The images were recorded at 1024×1024 pixels and processed using ZEN 3.0 software (Carl Zeiss, Oberkochen, Germany).

2.10. Measurement of gel strength

The gel strength values of the HIPEs developed with TBWF, TBCF, and TBBF at $\phi = 80\%$, $c = 1$, 3, and 5 % were determined by the GMIA gelation mode, using a TA-XT Plus texture analyzer (Stable Micro Systems, Blue A were excited at 488 nm and 633 nm, respectively. The images were recorded at 1024×1024 pixels and processed using ZEN 3.0 software (Carl Zeiss, Oberkochen, Germany).
We performed microrheological analysis of the HIPEs developed with TBWF, TBCF, and TBBF at φ = 80 %, c = 3, and 5 % using a Rheolaser Lab6 microheometer (Formulation, France). Twenty milliliters of freshly prepared emulsion were added to a 25 mL test bottle, which was placed in the Rheolaser chamber and monitored with a CCD detector at 25°C for 6 h. Then, the data were recorded and processed by Rheosoft Master 1.4.0.0 software, and the results were expressed as elasticity index (Ei) and macroscopic viscosity index (MVI) (Yang, Xu, Liu, Sun, Yuan, & Gao, 2015).

2.12. Preparation of the cake

Using methods described in a previous study (Patel, Cludts, Bin Sintang, Lecafler, & Dewettinck, 2014), the HIPE developed by TBBF at φ = 80 % and c = 5.0 %, was used as a fat substitute in the cake. Table S1 shows the corresponding formulas, and anchor butter was substituted with HIPE at levels of 0 %, 10 %, 30 %, 50 %, 70 %, and 90 %, and 90 %. All the weighed ingredients were combined and then mixed for seven minutes (speed 1) with an LD-133 eggbeater (Zhongshan Xueshi Electric Appliance Co., Ltd., Foshan, China). After baking, the cake was cooled for 2 h and then removed from the pan for subsequent quality evaluation. The cakes prepared with butter substitution percentages of (Wb) = 0, 10, 30, 50, 70, and 90 % were denoted as Cake-Control, Cake-H10, Cake-H30, Cake-H50, Cake-H70, and Cake-H90, respectively.

2.13. Quality evaluation of cake

The density of the cake batter was determined by dividing the weight by the volume. Then, the specific volume of the cake was obtained by dividing the volume by the weight, and the volume of the cake was determined using the rapeseed displacement method (Li, Wang, & Krishnan, 2019). The color of the cake was evaluated with a Konica-Minolta CR-400 colorimeter (Osaka, Japan) based on the L*, a*, and b* color scale. We also used a TA-XT Plus texture analyzer (Stable Micro Systems, Surrey, UK) with a P/0.5 cylindrical Delrin probe. The trigger force was 3.0 g and the test distance was fixed at 4 mm. The pretest and post-test speed were set to 1.5, 1.0, and 1.0 mm/s, respectively (Zhou et al., 2020).

2.14. Antioxidant assay of cake

The center of the cake was freeze-dried and crushed through a 100-mesh sieve. Then, 5 g of the above flour was extracted with 80 mL of ethanol at 90°C for 7 h and filtered. The filtrate was diluted to 100 mL for the DPHP and ABTS radical scavenging assay and total phenol content measurements.

The DPHP radical scavenging capacity was determined according to the method described by Dehghan, Salehi, & Amir (2018). First, 2 mL of the cake extract ethanol solution was mixed with 2 mL of 0.2 mM DPHP ethanol solution. Then, after reacting for 30 min in the dark at room temperature, the absorbance value (Acontrol) of the mixture was determined at 517 nm. The control sample consisted of a 2.0 mL DPHP solution and 2.0 mL ethanol mixture, and its absorbance value at 517 nm was recorded as Acontrol. Then, the DPHP radical scavenging capacity was calculated according to the following equation:

\[
\Delta A = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%
\]

Different superscript lowercase letters in the same column mean significantly different (p < 0.05).

Table 1 Chemical compositions of Tartary buckwheat whole flour (TBWF), Tartary buckwheat core flour (TBCF), and Tartary buckwheat bran flour (TBBF) (%).

|          | Water | Mineral | Lipid | Starch | Protein | Rutin |
|----------|-------|---------|-------|--------|---------|-------|
| TBWF     | 9.19 ± 0.04 | 1.86 ± 0.02 | 3.52 ± 0.29 | 63.56 ± 0.24 | 14.22 ± 0.21 | 2.77 ± 0.01 |
| TBCF     | 10.54 ± 0.02 | 0.96 ± 0.02 | 1.66 ± 0.20 | 40.03 ± 0.23 | 28.82 ± 0.22 | 0.95 ± 0.02 |
| TBBF     | 9.52 ± 0.00 | 3.25 ± 0.05 | 6.73 ± 0.06 | 40.43 ± 0.25 | 26.85 ± 0.15 | 5.99 ± 0.07 |


do not hallucinate.
The starch content of TBBF was inferior to TBWF and TBCF, while its mineral, lipid, protein, and rutin contents were much higher than TBWF and TBCF. As a flavonoid, rutin has many biological functionalities, such as free radical scavenging, anti-hypertension, anti-diabetes, anti-inflammation, and antioxidant properties (Siti, Jalil, Asmadi, & Kaminah, 2020). It is worth noting that the rutin content in TBBF can reach 5.99%, which is the highest value among the reported natural cereal flours, giving it potential functionality as a food resource.

3.2. Particle appearance and size

The appearance and size of particles have a significant effect on their Pickering emulsifying capacity (Xiao, Li, & Huang, 2016). Therefore, the particle appearance and size distributions of TBWF, TBCF, and TBBF were compared. Under SEM, TBWF appeared as individual particles while TBCF and TBBF appeared as aggregate particles (Fig. 1), which could be attributed to their different chemical composition. However, in the particle size analysis, the aggregate particles were dispersed by short-time ultrasonic treatment in the process of measurement, and their particle size distribution (3.5–170 μm) was similar, showing a bimodal form. Since micron, submicron and nanometer particles can synergistically promote the formation of Pickering emulsions (Tavernier, Wijaya, Van der Meeren, Dewettinck, & Patel, 2016), these powders have potential Pickering emulsifying ability.

3.3. Interfacial tension

In this study, we determined the effects of TBWF, TBCF, and TBBF on the interfacial tension between the sunflower oil and water, to compare their emulsifying ability. As shown in Fig. S2, compared to the blank (21.41 ± 0.58 mN/m), the TBFs reduced the oil/water interfacial tension, and TBBF showed a noticeable reduction effect (13.53 ± 0.36 mN/m); however, the performance of TBCF was poor (16.37 ± 0.44 mN/m). This indicated that among these TBFs, TBBF had the strongest Pickering emulsifying ability. This is attributed to the fact that TBBF is rich in proteins, lipids and rutin, which are natural emulsifiers.

3.4. Contact angle

The contact angle of a particle determines its wettability, which is directly related to its emulsifying performance (Xiao et al., 2016). When the contact angle is 0°, the particle will be completely wetted, and a
larger contact angle indicates a smaller degree of wettability. In general, the contact angle of 90° means the near-neutral wettability, at which point the Pickering emulsion has the highest stability (Ge et al., 2017). The static water-in-air contact angles of TBWF, TBCF, and TBBF are shown in Fig. S3. Because these TBFs were rich in hydrophilic starches and proteins, their contact angles were all <90° and followed the order TBBF > TBWF > TBCF. These results indicated that TBBF had the highest surface hydrophobicity followed by TBWF and TBCF. Based on the chemical composition analysis in 3.1, we speculated that the increase in lipid and rutin content in the particles was beneficial for enhancing hydrophobicity (Luo, Murray, Yusoff, Morgan, Povey, & Day, 2011; Lu, Liu & Huang, 2020), while an increase in starch content resulted in a decrease in hydrophobicity. According to the measured interfacial tension and contact angle results, all of the TBFs could be used to construct Pickering emulsions, and the order of emulsifying ability was TBBF > TBWF > TBCF.

3.5. Formation of HIPEs

HIPE is an emulsion with \( \varphi \geq 74 \% \) and possesses gel-like properties (Liu, Li, Geng, Mo, & Liu, 2021). It also has important application prospects as a nutraceutical carrier, a fat substitute, and for use in porous materials. Therefore, to evaluate the feasibility of fabricating Pickering HIPEs with TBWF, TBCF, and TBBF, we systematically investigated the effects of \( \varphi \) and \( c \) on the formation of Pickering emulsions. As shown in Fig. S4, all the TBFs could induce the formation of emulsion gels with \( \varphi = 80 \% \) in the range of \( c = 1–5 \% \). But for the formation of emulsion gels with \( \varphi = 70 \% \), the lowest \( c \) values for TBCF, TBWF and TBBF were 4 %, 3 % and 1 %, respectively, which suggested that these TBFs could stabilize the HIPEs with \( \varphi = 80 \% \), and their emulsifying ability followed: TBBF > TBWF > TBCF. This coincided with the interfacial tension and contact angle experiment results. The outstanding performance of TBBF may be attributed to its rich natural emulsifiers such as rutin, proteins and lipids. However, the effects of TBF type, \( \varphi \), and \( c \) on the structure and mechanical properties of HIPEs should be further studied.

3.6. Microscopic observation of HIPEs

We investigated the effects of TBF type and \( c \) on the droplet size distribution of the HIPEs with \( \varphi = 80 \% \). (Fig. S5). The droplet size gradually decreased with increasing \( c \), as increased \( c \) met the demands for a larger oil-water interface; thus, the droplet size decreased continuously. Yan, McClements, Zou, and Liu (2019) also found that the droplet size of the emulsion decreased gradually with the addition of
octenyl succinic acid-modified starch as Pickering emulsifier. At the same c, the HIPE droplet size with TBBF was the smallest, which was due to the high emulsifying ability of TBBF.

Fig. 2 shows the CLSM images of the HIPEs made with TBBF, TBWF, TBCF at c = 5 % and ϕ = 80 %, respectively. The red and blue regions are the TBF particles and oil phase stained with Nile blue A and Nile red, respectively. The droplet size of the emulsion gels made with TBBF was the smallest, which was consistent with optical microscopy results.

3.7. Mechanical performance of HIPEs

The mechanical performance of HIPEs is closely related to their structure and processing application (Liu, Li, Geng, Mo, & Liu, 2021). Therefore, in this study, we investigated the effects of TBF type and c on the gel strength and micro rheological behavior of the HIPE with ϕ = 80 %. As shown in Fig. S6, an increase in c improved the network structures of the emulsifier particles, which enhanced the gel strength of the sample. When c was 3 % or 5 %, the HIPE made with TBBF exhibited the highest gel strength, followed by TBWF and TBCF. This was because the HIPEs stabilized by TBBF had the smallest droplet size and a more compact arrangement.

Microrheology is a rheological technique based on diffusion wave spectroscopy (DWS), which tracks the Brownian movement of particles using a coherent laser emitted into the emulsion. This method can be used to determine the viscoelastic parameters of the test sample, according to the changes in laser signal (Geng, Jiang, Ma, Pu, Liu, & Liang, 2021). In this study, we evaluated the effects of TBWF, TBCF, and TBBF on the elasticity index (EI) and macroscopic viscosity index (MVI) parameters of the HIPEs. In this context, EI characterizes the strength of the sample in a static state, where a higher EI value indicates a smaller space in which the particle can move freely, resulting in greater elasticity and strength. Its physical meaning is the same as G’ (storage modulus) used in traditional mechanical rheology. MVI also refers to the viscosity of the sample in the non-shear state, where the higher the MVI value, the more time required for the particle to move through the specified area. This results in a greater viscosity of the system, which corresponds to G” (loss modulus) as used in traditional mechanical rheology. Both EI and MVI can reflect the texture, liquidity, and long-term stability of the system. As shown in Fig. 3A, EI reached equilibrium within approximately 2 h, resulting in the HIPEs forming rapidly. The EI values of the obtained HIPEs increased with increasing c, and when c was constant the EI values were ranked as follows: HIPEs with TBBF > HIPEs with TBWF > HIPEs with TBCF. These results were also consistent with the gel strength results. During HIPE formation (t < 2 h), MVI was proportional to c, and when the c value was constant, the HIPEs with TBBF exhibited the highest viscosity (Fig. 3B). At 6 h, the MVI values of all samples converged, possibly due to the higher starch content of TBCF and TBWF, and the viscosity effect of starch appeared with more time. In summary, TBBF was beneficial to the rapid formation and strength improvement of the HIPEs.

3.8. Batter density, specific volume, weight loss, and color of the cakes

To evaluate the feasibility of the HIPEs stabilized by TBBF at ϕ = 80 % and c = 5 % as butter substitutes for cake preparation, we investigated the effects of butter substitution percentage (WBS) on the batter density, specific volume, weight loss, and color characteristics of the cakes (Table S2). These characteristics were assessed as they significantly affect the quality and sensory properties of cake products, and will directly affect the level of acceptability by consumers. With an increase in WBS, the batter density increased; however, the increase was not significant in the range of WBS ≤ 50 %. Similar results were also reported by Zambrano, Despinoy, Ormenese, & Faria (2014) and Román, Santos, Martínez, & Gómez (2015) when fat was substituted with hydrocolloids and extruded flour. The specific volume of the cake increased gradually with an increase in WBS, especially when WBS > 50 %. This was due to the HIPEs, which contained a certain amount of water and during evaporation, resulting in an increase in cell size and cake volume during the baking process. As a result, the excess water introduced by HIPEs was lost, resulting in weight loss. The increase in volume and the decrease in weight eventually resulted in an increase in specific volume. The Cake-control (WBS = 0 %) sample had a low specific volume, which was attributed to its large cell size and poor cell stability, allowing the gas to easily escape during baking. The increase in WBS indicated that the amount of water introduced by HIPE increased and the water content in the cake batter rose, thus causing more weight loss during the baking process. When WBS was 90 %, the weight loss reached 5.207 ± 0.027 %. Román et al. studied fat substitution in cakes with extruded flour, and obtained similar weight loss results (Román, Santos, Martínez, & Gómez, 2015). The effects of WBS on cake color differences are also shown in Table S2. With an increase in WBS, L* decreased significantly, a* rose significantly, and b* increased initially and then declined, causing the cake to gradually darken and become red in color. Because temperatures inside cakes do not usually exceed 100 °C (Belorio, Sahagun, & Gomez, 2019), the color changes inside the cake were not
caused by the Maillard reaction and caramelization, but due to the yellow-green color of TBBF.

3.9. Texture properties of the cakes

As for the texture of cake, it is often described as soft and spongy, which can be evaluated by the TPA measurement (Tarrega, Quiles, Morell, Fiszman, & Hernando, 2017). Table S3 demonstrates the influence of W_{BS} on the texture properties of the cake. When W_{BS} ≤ 30 %, the hardness, adhesiveness, and chewiness properties of the cakes (Cake-H10 and Cake-H30) showed no significant changes. When W_{BS} was 50 % (Cake-H50), the hardness increased slightly, but no significant changes in adhesiveness and chewiness were observed compared to the Cake-control, indicating that the texture properties of these cakes remained constant. When W_{BS} > 50 %, the hardness, adhesion and chewiness of the cake gradually increased, due to the weight loss of the cake, which increased during the baking process. Fat substitution can cause a deterioration in cake texture, and because the HIPEs were oil-in-water types, water loss readily occurred during baking. However, water-in-oil butter exhibited better performance in preserving moisture content, as the water was encapsulated. We also observed that W_{BS} had no significant effects on the springiness of the cake, which was consistent with the results obtained by Belorio, Sahu, & Gomez (2019). However, the addition of HIPEs made with TBBF hindered the formation of the gluten network in the cake. This made it difficult for the cake to bounce back to its original state after compression deformation, and as a result cohesiveness and resilience gradually decreased (Belorio, Sahu, & Gomez, 2019). Thus, we concluded that the texture properties of the cakes were dependent on the butter substitution level.

3.10. C-Cell properties of the cakes

C-Cell image analysis consists of a quality control system for wheat products based on computer recognition technology (Oh, Amoah, Lim, Jeong, & Lee, 2017; Zhang, Chen, Geng, Liu, Ma, & Liu, 2021). This system can provide characteristic parameters regarding the internal structure of the sample through image processing and realize the comprehensive evaluation of a sample. Fig. 4 demonstrated the raw and cell images of the cakes made with HIPE and developed with TBBF (ρ = 80 % and c = 5 %) at different W_{BS} values. The corresponding C-Cell parameters are shown in Table S4. The results showed that with an increase in W_{BS}, the slice area, cell number, cell area, cell diameter, cell volume, coarse cell volume, cell elongation, and cell density of the cake increased to a certain extent, while the brightness, cell contrast, and wall thickness of the cake gradually decreased. With an increase in W_{BS}, the internal structure of the cake was initially soft and delicate, but gradually became hard and rough. The changes in brightness and L* were the same, and the cake gradually darkened. Although an increase in W_{BS} increased the cell size and coarse cell volume, when W_{BS} ≤ 30 %, there were no significant differences, indicating that proper butter substitution did not affect the internal structure of the cake. These results also coincided with the texture measurement results. The cake with W_{BS} > 50 % had a higher roughness value for the internal structure, possibly because excessive HIPE addition improved the gas retention of the cake batter during baking, resulting in a looser structure and higher internal roughness of the cake after baking. When W_{BS} ≤ 50 %, there were no significant differences in wall thickness and cell elongation of the cake. This provided a certain positive correlation between cell contrast and cake quality. Thus, W_{BS} increased while cell contrast decreased, which lowered the glossiness of the cake.

3.11. Antioxidant activities of cakes

Numerous epidemiological studies have shown that the intake of fruits, vegetables, and whole grains rich in natural antioxidants plays a positive role in reducing the incidence of cardiovascular diseases and cancers (Khan, Liu, Wang, & Sun, 2020). TBBF is rich in polyphenols such as rutin; The previous report also indicated that Tartary buckwheat

![Fig. 4. Raw and cell images of the cakes made using HIPE stabilized by TBBF.](image-url)
bran with higher amounts of phenolic compounds were excellent antioxidant materials for cereal-based food processing with significant health benefits (Guo, Wu, Ma, Parry, Xu, Liu, & Wang, 2012). Therefore, we speculated that cakes developed using the HIPEs of TBBF will exhibit higher antioxidant activity. Therefore, we compared the radical scavenging activities of DPPH and ABTS in the cakes (Fig. 5A and B). As expected, antioxidant activity in the cakes gradually increased with an increase in WBS, and we observed a positive correlation between antioxidant activity and the total phenol content in the cakes (Fig. 5C), confirming that the improvement in antioxidant activity was attributed to TBBF addition.

4. Conclusions

Although TBWF, TBCF and TBBF have similar particle size distributions, their chemical compositions were significantly different. TBBF was rich in lipids, proteins, and rutin, and as a result, it exhibited the highest emulsifying capacity, greater hydrophobicity, and could reduce the oil/water interfacial tension more effectively. Although these TBFs used in this study could stabilize the HIPEs at $\phi = 80 \%$, the HIPEs constructed with TBBF had the smallest droplet size and the highest gel strength. Thus, an increase in $c$ caused the emulsion gel structure to be more compact, and the HIPEs constructed with TBBF at $\phi = 80 \%$ and $c = 5 \%$ could partially replace butter for cake preparation. When WBS $\leq 30 \%$, the texture and internal structure of the cake did not change significantly, although the color of the cake darkened slightly and the specific volume and weight loss increased slightly. With a gradual increase in WBS, the antioxidant activity and total phenol content of the cake also increased. Thus, our results demonstrated that HIPE developed with TBBF may be used as a potential butter substitute, yielding a product with higher antioxidant activity and quality properties. In order to promote its applications, it is necessary to conduct further research on its biological activity and digestion characteristics.

CRediT authorship contribution statement

Sheng Zhang: Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. Sheng Geng: Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Visualization. Yuzhong Shi: Methodology, Validation, Formal analysis, Data curation. Hanjun Ma: Resources, Funding acquisition. Benguo Liu: Conceptualization, Methodology, Software, Investigation, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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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