Effect of Physiochemical Factors and Peanut Varieties on the Charge Stability of Oil Bodies Extracted by Aqueous Method

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Abstract: In order to explore the scientific basis for the application of oil bodies (OBs) from different peanut varieties in food, the effect of NaCl (0–100 mM), thermal processing (25–45 °C, 1 h) and pH (3.0, 7.4, and 9.0) on their zeta potentials was analyzed in this study. The zeta potentials of OB suspensions (in 10 mM phosphate buffer) prepared from five peanut varieties in different salt concentrations (0–100 mM) were positive at pH 3.0, while they remained negative at pH 7.4 and 9.0. The absolute values of zeta potentials were over 20 mV at a lower salt concentration (< 10 mM NaCl) at pH 3.0 and 7.4. Particularly, the values of zeta potentials of Yuhua27 and Yuhua9830 were as high as 40 mV in the absence of NaCl at pH 7.4. The OBs exhibited diverse change trends between the five peanut varieties in the temperatures from 25 to 45 °C (0 mM NaCl, pH 7.4). The OBs from Yuhua9830 exhibited the best thermal adaptability at the different temperatures tested than the other four peanut varieties. These outcomes suggested that OBs extracted from different varieties possess diverse properties and may provide a new insight into choosing a suitable peanut variety for the food industry.

Key words: different peanut varieties, oil bodies, charge stability, Zeta potential

1 Introduction

Peanut is one of the major oilseed sources in the world, providing nutritional components such as vegetable protein, carbohydrates, vitamins, minerals, fiber and oil for millions of people. In China, the total output of peanuts reached 17,500 thousand metric tons in 2017, which is at the leading position globally. Moreover, obvious component differences exist due to the cultivation of over 300 different varieties of peanuts across the world. As the competition from other crops has been increasing, the Peanut Foundation (TPF) in the United States solicited general proposals to address research priorities relevant to the needs of the peanut industry for 2017. Among these proposals, Variety Development and Crop Production Practices were one of the most important research priorities, which indicated that new properties may account for the greater adaptability relevant to not only peanut planting but also food processing.

The lipids of peanut, mainly triacylglycerols which contain high amounts of mono- and poly-unsaturated fatty acids, exist in minute and distinct intracellular organelles called ‘oil bodies’ (OBs). An OB consists of a neutral lipid matrix core, which is coated by a phospholipid monolayer embedded with OB intrinsic proteins. In recent years, OBs have attracted much attention owing to their useful characteristics i.e., their ability to exist as distinct entities and act as emulsifying agents for an extensive range of food products, from imitation milk and yogurt to ice cream.

Due to the mixed layer of phospholipids and hydrophobic proteins, OBs demonstrate exceptional physical and chemical stability against the stressful environmental factors to which they are generally exposed. Stability is crucial to the effective application of OBs, and it is closely related to many factors, such as the charge of particles, the size of particles, molecular configuration, and interfacial tension. In previous studies, many researchers have investigated the influence factors such as pH, ionic strength, and temperature on the stability of OBs obtained from different oilseeds. In these studies, zeta potential has been widely applied as an essential indicator to evaluate the charge stability of an OB. If the absolute zeta potential of the suspended particles was high, it indicated that these particles were excluded by each other and did not tend to flocculate. Although OBs from a number of
sources have been currently in use, there is only a handful of literature characterizing OBs prepared from different sources.

In order to explore possible differences among varieties of OBs and utilizing the most favorable variety, their characteristics must be understood. In the present work, thus, the zeta potential of the surface shear layer of OBs extracted from five peanut varieties was measured under different conditions of pH, ionic strength, and temperature for the purpose of providing new insights into choosing a suitable peanut variety for application in the food industry.

2 Materials and methods

2.1 Materials

Peanuts of five varieties (Yuhua23, Yuhua27, Yuhua9719, Yuhua9830, and Yuhua9502 harvested in August 2016 from Henan province, China) were supplied by Henan Academy of Agricultural Sciences and stored at 4°C until use. Chemical reagents were of analytical grade and were acquired from Sinpharm Chemical Reagent Co. Ltd. (Shanghai, China). Water from a Millipore Milli-Q water purification system (≥18.2 MΩ) was employed in all runs.

2.2 Characterization of peanut compositions

The proximate analysis of the five peanut varieties (Yuhua23, Yuhua27, Yuhua9719, Yuhua9830, and Yuhua9502) used in this study was carried out. The moisture content was estimated through oven drying (105°C for 3 h), the ash in the muffle furnace (500°C for 5 h), the lipid content through the Soxhlet process (hexane extraction for 13 h), and the protein content via the Kjeldahl technique.

2.3 Isolation of peanut OBs

OBs were isolated by means of an aqueous extraction process with some modifications. In brief, the dehulled peanut seeds were peeled, and then a 20 g sample of crushed peanuts was transferred to a beaker and dispersed in deionized water at 1:5 (w/v) seed-to-water ratio. The mixture was stirred by the Fluko homogenizer (18000 rpm, FM200) for 10 s and then incubated for 1 h at 45°C in a constant temperature bath shaker (THZ-82, Huafeng, Tianjin, China). Subsequent to filtration through four layers of gauze cloth, the filtrate was centrifuged (DZ267-32C6, Anting, Shanghai, China) at 4000 rpm for 30 min. The OBs were obtained as a creamy pad at the topside of the centrifuge tube and were stored at 4°C for no longer than 24 h.

2.4 Determination of protein concentrations through amino acid analysis

Briefly, each sample (0.5 g) was hydrolyzed with 4 mL of conc. HCl (12 M) at 110°C for 22 h in a hydrolysis tube. The amino acid content was determined according to Ying and others using High-performance liquid chromatography (Waters 2695, with Waters amino acid analysis package, Waters, America) [16]. Further, the overall amino acid content was utilized to estimate protein concentrations in each sample.

2.5 Determination of phospholipid and phospholipid fatty acid composition of peanut OBs

The phospholipid content was determined by the molybdenum blue colorimetric method and carried out according to the AOCS Official Method Ca 12-55 with minor modifications. On the other hand, the determination of phospholipid fatty acids was in accordance with the Chinese national standard method (GB5009.168-2016).

2.6 Preparation of OB suspensions for zeta potential analysis

The effects of pH, ionic strength and temperature on the stability of OBs obtained from five peanut varieties were evaluated according to a previously reported method with minor modifications [15]. OB suspensions were prepared by adding 1 g of cream to 9 g of 10 mM Na2HPO4-NaCl buffer solution (0, 10, 40, 80, and 100 mM) at pH 3.0, 7.4 and 9.0, which was subsequently incubated at 37°C for 20 min and stored at 25°C for 24 h prior to zeta potential analysis.

To study the effect of thermal processing, the suspensions were synthesized by adding 1 g of cream to 9 g of 10 mM Na2HPO4 buffer solution at pH 7.4, which was then incubated at a specific temperature (25, 30, 35, 40, and 45°C for 1 h). Subsequently, it was cooled to room temperature and stored at 25°C for 24 h before zeta potential analysis.

2.7 SDS-PAGE

SDS-PAGE was carried out according to Laemmli [17]. The concentrations of the stacking and separating gels being 5% and 12%, respectively. Simply, the OB was diluted with sample buffer to a protein concentration of 2 mg/mL, heated at 100°C for 5 min, and then cooled to room temperature. Then 10 μL was loaded into sample well. The electrophoresis was carried out at constant current of 40 mA until all samples entered into the separation gell, and then at a constant current of 30 mV until the end. The gel was stained using coomassie brilliant blue G-250.

2.8 Zeta potential analysis

Suspensions prepared from the OBs of five peanut varieties using different treatments were diluted to about 0.05 (w/v) concentrations. The diluted suspensions were measured directly by the Zeta Potential Analyzer instrument (Brookhaven Instruments Corporation, America).

2.9 Statistical analysis

All experiments were performed in duplicate, and the data were analyzed for the variance using analysis of
variance (ANOVA) and were represented as mean ± standard deviation (SD). $P < 0.01$ was considered as significant. Statistical analysis of the acquired data was carried out using the SPSS 22.0 software.

3 Results and discussion
3.1 Composition analysis of different peanut varieties

In China, the cultivation areas of peanut are the Yellow River basin, the Yangtze River basin, southeast coastal areas, Yunnan-Guizhou Plateau, Loess Plateau, northeast region, and northwest region. Furthermore, more than 80% of production in China takes places in eight provinces: Henan, Shandong, Hebei, Guangdong, Anhui, Sichuan, Liaoning and Guangxi (Table 1). Henan ranks as the largest peanut producer in China, which is located in the Yellow River basin. There are mainly four classes of peanuts in Henan: Yuhua, Kainong, Puhua, and Yuanza series. Among these four classes, Yuhua series is the most widely grown in the Henan province. In this work, the five common Yuhua varieties (Yuhua23, Yuhua27, Yuhua9719, Yuhua9830, and Yuhua9502) were selected in order to discuss the stability of OBs between peanut varieties, while the average values of lipid, protein, and ash from these varieties are shown in Table 2. The outcomes revealed that the protein content ranged from 21.1% to 26.5%, the lipid varied from 41.3% to 48.7% and the ash content was between 2.5% and 3.5%. Meanwhile, the results of variance analysis indicated that there existed obvious differences in lipid, protein, and ash content ($p < 0.01$) between peanut varieties.

3.2 The protein and phospholipid components of different peanut OBs

The SDS-PAGE results of different peanut OBs are shown in Fig. 1. The protein molecular weight distribution of the five peanut OBs was basically the same, and all contained 15 to 26 kDa (oleosin), 27 kDa (caleosin), and 29.4 kDa (proteins). The data were expressed as means ± standard deviations. The data in a column marked with diverse capital letters were significantly different ($p < 0.01$).

Table 2 Composition of five peanut varieties (g/100 g dry matter).

| Peanut varieties | Lipid     | Protein    | Ash       |
|------------------|-----------|------------|-----------|
| Yuhua23          | 45.4 ± 1.0 B | 22.5 ± 0.3 B | 2.5 ± 0.05 C |
| Yuhua27          | 43.9 ± 0.8 C | 22.0 ± 0.5 B | 2.7 ± 0.05 B |
| Yuhua9719        | 41.3 ± 0.8 D | 26.5 ± 0.6 A | 2.5 ± 0.04 C |
| Yuhua9830        | 48.7 ± 0.9 A | 21.1 ± 0.4 C | 3.5 ± 0.06 A |
| Yuhua9502        | 48.3 ± 1.0 A | 22.5 ± 0.4 B | 2.7 ± 0.04 B |

Table 1 Main peanut cultivation regions in China.

| Province | Cultivated area (million hectares) | Yield (million metric tons) | Per unit area yield (kg/ha) |
|----------|------------------------------------|-----------------------------|-----------------------------|
| Henan    | 1.0583                             | 4.7129                      | 4453.3                      |
| Shandong | 0.7553                             | 3.3130                      | 4386.3                      |
| Hebei    | 0.3525                             | 1.2924                      | 3666.4                      |
| Guangdong| 0.3574                             | 1.0431                      | 2918.6                      |
| Anhui    | 0.1904                             | 0.9435                      | 4955.4                      |
| Sichuan  | 0.2611                             | 0.6665                      | 2552.7                      |
| Liaoning | 0.3056                             | 0.6204                      | 2030.1                      |
| Guangxi  | 0.2043                             | 0.5757                      | 2817.9                      |
The amino acid contents of the OB proteins extracted from five peanut varieties are shown in Table 3. The amino acid content of Yuhua27 was the lowest, while that of Yuhua23 was the highest. Although Yuhua27 and Yuhua9830 possessed a lower amino acid content, they displayed better charge stability at different salt concentrations and pH values (Fig. 2), it might be due to Yuhua27 and Yuhua9830 have less extrinsic protein embedded on their surface. As we all know, there are some intrinsic protein (oleosin, calseolin, steroleosin) on the peanut oil body, which have an important role on stability of oil body. However, in aqueous extraction, OBs will inevitably adsorb some extrinsic proteins, and the more extrinsic proteins were bound to OBs, the more easily the heat-induced coalescence of OBs occurred. Therefore the lower amino acid (protein) content may mean higher ratio of oleosin on the OB surface which may enhance the stability of OBs. The glutamic acid content of OBs extracted from Yuhua23, Yuhua9719, Yuhua9830 and Yuhua9502 were the highest and they accounted for 14.1%, 14.3%, 13.6% and 14.1%, respectively. Particularly, the glutamic acid content of Yuhua27 was the same as that of the threonine content, accounting for 12.8%. Subsequently, the threonine content of Yuhua9830 and Yuhua9502 was the second-ranked one; meanwhile, the aspartic acid content of Yuhua23, Yuhua27 and Yuhua9719 was the second-ranked. Moreover, the mass fraction of polar amino acids of OBs extracted from the five peanut varieties was higher than non-polar amino acids, which were beneficial to form a stable o/w emulsion.

The OB phospholipid contents of Yuhua23, Yuhua27, Yuhua9719, Yuhua9830 and Yuhua9502 were 5.08%.
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5.40%, 5.26%, 5.30% and 5.53%, respectively (figure not shown). Similar to the low phospholipid content of Yuhua27, the phospholipid content of the other four peanut varieties displayed basically no difference ($p < 0.05$). The fatty acid fractions from the five peanut OBs are shown in Table 4. Yuhua9719 and Yuhua9502 possessed eight kinds of fatty acids, whereas Yuhua23, Yuhua27 and Yuhua9830 only possessed six. The total fatty acids were dominated by palmitic, linoleic and oleic acids, with other fatty acids only accounting for a small fraction. Especially, the oleic acid contents of Yuhua23, Yuhua27, Yuhua9719, Yuhua9830 and Yuhua9502 were the highest and accounted for 38.78%, 41.75%, 43.93%, 39.64% and 40.74%, respectively, which was comparable to the fatty acid composition of peanut oil$^{30}$. Peanut OB phospholipids were composed of a greater percentage of unsaturated fatty acids and a lower percentage of saturated fatty acids. The high content of unsaturated fatty acids can increase the membrane fluidity and may be beneficial to the stability of the OB$^{31}$. Several researchers have suggested that saturated fatty acids contribute to the formation of large OBs which may influence the stability of the OB$^{32}$. Thus, further experiments are still needed to investigate this connection.

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Fig. 2 Zeta potential of oil bodies extracted from different peanut varieties (a) Yuhua23, (b) Yuhua27, (c) Yuhua9502, (d) Yuhua9719, (e) Yuhua9830 at different salt concentrations and pH values, where error bar represents standard deviation and different capital letters were significantly ($p < 0.01$) different.
3.3 Influence of physicochemical factors on the stability of different peanut OBs

3.3.1 pH and NaCl

Zeta potentials could be used as indicators of the stability of OB suspensions\(^35\). Guidelines were generally used, which classified suspensions with zeta potential values of \(\pm (0-10)\), \(\pm (10-20)\), \(\pm (20-30)\) and \(> \pm 30\) mV as extremely unstable, relatively stable, moderately stable and extremely stable, respectively\(^36\). The zeta potentials of the OBs extracted from five peanut varieties remained positive at pH 3.0 (Figs. 2a-2e). On the other hand, these values remained negative at pH 7.4 and 9.0. These results indicated that the charge property of OBs was affected by pH, possibly because the amino acid residues at the N- and C-termini of the surface of OBs are affected by pH\(^37\). Moreover, all the five peanut varieties have higher levels of polar amino acids than the non-polar amino acids, and the amount of negatively charged amino acids was higher than that of the positively charged ones. Consequently, OBs have a net positive charge under acidic conditions and a net negative charge in neutral or alkaline environments.

In this experiment, the five peanut varieties had different charge levels under pH of 3.0, 7.4, and 9.0, which indicates the charge stability of different peanut oil bodies is different. The surface charge of OB has been attributed to the orientation of intrinsic protein on the surface of the OB\(^38, 39\). The positively charged amino acids of the OB protein are thought to interact with the negatively charged PE and PI on the phospholipid monolayer allowing the negatively charged amino acids of the OB protein to face outwards giving an overall negative charge\(^40\). And the stability of OBs mainly depend on their intrinsic protein and phospholipids\(^40\), so difference peanut varieties contain different intrinsic protein and phospholipids may lead to the difference of their charge stability.

With the increase in NaCl concentration at diverse pH ranges, the zeta potentials of OBs from all the five peanut varieties declined differently. A high salt concentration resulted in a distinctly lower stability at pH 3.0, 7.4, and 9.0. At pH 3.0, the OBs of Yuhua23 displayed better charge stability than others. The maximum zeta potential value of these peanut varieties appears under the condition of 0 mM NaCl at pH 7.4. Particularly, the zeta potentials of Yuhua27 and Yuhua9830 were as high as -40 mV. At the same time, the increase in NaCl concentrations has a higher significant impact on the decrease of the zeta potentials of OBs from all five peanut varieties at pH 7.4 than pH 3.0 and 9.0. At pH 9.0, when the salt concentration was increased from 0 to 10 mM, the zeta potential of Yuhua9502 increased slightly from 18.8 mV to 22.1 mV and then declined weakly. There was no notable difference in the zeta potential of Yuhua9502 in the concentration range of 0–80 mM NaCl. These results indicated that there was a decrease in the zeta potential with the increasing NaCl concentration and that OBs of all five peanut varieties were stable to aggregation at low NaCl concentrations\(< 10\) mM). Sukhotu and others\(^47\) found that at pH 7.0, increasing the concentration of NaCl(0-100 mM) will lead to the decrease of zeta potential, but continue to increase the concentration of NaCl(>100 mM) only have a slight effect on the charges of OB suspensions. In this research, Yuhua27 and Yuhua9830 exhibited high stability (zeta potential >35

### Table 4 Analysis of fatty acid contents of different peanut varieties (FA/TFA/\(\%\)).

| Name of FA | Yuhua23 | Yuhua27 | Yuhua9830 | Yuhua9719 | Yuhua9502 |
|-----------|---------|---------|-----------|-----------|-----------|
| C16:0     | 15.60 ± 0.02 A | 13.98 ± 0.05 C | 11.96 ± 0.03 E | 15.29 ± 0.02 B | 12.56 ± 0.00 D |
| C18:0     | 5.35 ± 0.00 A | 5.10 ± 0.05 B | 4.65 ± 0.00 C | 4.18 ± 0.03 D | 4.72 ± 0.01 C |
| C18:1     | 38.79 ± 0.19 E | 41.75 ± 0.06 B | 39.64 ± 0.03 D | 43.93 ± 0.35 A | 40.74 ± 0.01 C |
| C18:2     | 37.13 ± 0.15 B | 35.65 ± 0.08 D | 37.81 ± 0.00 A | 33.69 ± 0.16 E | 36.38 ± 0.00 C |
| C20:0     | 1.29 ± 0.07 B | 1.59 ± 0.10 A | 1.79 ± 0.00 A | 1.18 ± 0.08 B | 1.75 ± 0.01 A |
| C20:1     | ND       | ND       | 0.70 ± 0.01 A | ND       | 0.63 ± 0.00 B |
| C22:0     | 1.85 ± 0.01 B | 1.93 ± 0.03 BC | 2.42 ± 0.00 A | 1.73 ± 0.07 C | 2.31 ± 0.00 A |
| C24:0     | ND       | ND       | 1.04 ± 0.01 A | ND       | 0.92 ± 0.00 B |
| O/L       | 1.04 ± 0.01 D | 1.17 ± 0.00 B | 1.3 ± 0.00 A | 1.05 ± 0.02 D | 1.12 ± 0.00 C |
| SFA (%)   | 24.08 ± 0.04 A | 22.59 ± 0.02 B | 22.38 ± 0.03 B | 21.86 ± 0.19 C | 22.26 ± 0.01 B |
| UFA (%)   | 75.92 ± 0.04 C | 77.41 ± 0.02 B | 77.62 ± 0.03 B | 78.14 ± 0.19 A | 77.74 ± 0.01 B |
| SFA/UFA   | 0.32       | 0.29     | 0.29       | 0.28       | 0.29       |

Data were expressed as means ± standard deviations. The data in a column marked with diverse capital letters were significantly \((p < 0.01)\) different. FA: Fatty Acid; TFA: Total Fatty Acid; SFA: Saturated Fatty Acid; UFA: Unsaturated Fatty Acid; ND: not detected.
The peanut OBs were easily affected by salt and the aggregation of oil bodies caused by hydrophobic interactions of proteins were easily formed\(^{(27)}\). Similar results were obtained for soybean OBs that were not coated by \(\kappa\)-carrageenan\(^{(37)}\), and in OB suspension from maize germ in the absence of SDS\(^{(35)}\). This decrease in zeta potential in the concentration range of 0–100 mM NaCl might be due to electrostatic screening effect and to a decrease in the electrostatic interaction energy produced by the salt\(^{(39, 40)}\). The insensitivity of the zeta potential of OBs from some peanut varieties to the addition of NaCl may be attributed to the incidence of natural salts in the peanut cells. For instance, adding monovalent cations might dislocate divalent cations associated with the anionic surfaces of OBs at neutral pH to some extent, thus counterbalancing the anticipated decline in negative charge\(^{(40)}\). Thus, a low salt concentration can benefit the solubility of the surface proteins of OBs in the aqueous media and enhance the stability of OBs, although increased salt concentrations have an opposite effect on the stability due to the weak protein hydration\(^{(39)}\).

### 3.3.2 Temperature

For the utility of OBs in food processing, it is important to study the effect of temperature on the zeta potential of the OB suspensions (Table 5). A pronounced difference existed in the electrical charge of the OBs extracted from the five peanut varieties when their suspensions were heated; the zeta potential of Yuhua27 decreased, while that of the others increased. Different peanut varieties showed various magnitudes of the electrical charge on the OB in the range of temperatures from 25 to 45°C. As for Yuhua23, the zeta potential increased from around \(-30.40 \pm 2.87\) mV at 25°C to the maximum value of \(-39.98 \pm 3.41\) mV at 30°C, and then it did not change significantly further. Additionally, Yuhua9719 and Yuhua9830 displayed the same change trend as that of Yuhua23, and the peak zeta potentials of which were \(-42.10 \pm 3.46\) mV at 40°C and \(-46.88 \pm 2.44\) mV at 35°C, respectively. Nevertheless, the zeta potential of OBs extracted from Yuhua27 was reduced from about \(-40.21 \pm 2.73\) mV with the increase in temperature from 25 to 45°C, while the zeta potential of Yuhua9502 followed a negative trend from \(-38.03 \pm 2.09\) mV.

As shown in Table 3, Yuhua23, Yuhua9719, and Yuhua9830 exhibited more persistent charge stability (zeta potential > 30 mV) within 25–45°C than Yuhua27 and Yuhua9502. Additionally, the zeta potential of Yuhua27 and Yuhua9502 showed an abrupt change trend, but the other three varieties exhibited a mild change. From a thermodynamical point of view, various types of instability mechanisms (Ostwald ripening, creaming, aggregation, partial coalescence, and coalescence) are the basis of alterations in dimensions, quantity, and arrangements of droplets in oil-in-water emulsions\(^{(41)}\). The obvious difference in zeta potentials between the OBs of the five peanut varieties at 25–45°C partly depended on the mean particle diameter of the OBs, the ratio of oleosin and phospholipids on the surface layer of OBs, and the interactions between them. Moreover, this difference may be due to the discrepancy in the composition and/or structure of the oleosin, caleosin, and stereosin on the interfacial layer of OBs\(^{(10)}\). The OBs extracted from Yuhua9830 displayed the best thermal adaptability (there was no more significant difference from the other four peanut varieties), which may be due to a wider range of size distributions and a higher content of non-thermosensitivity protein than the other four varieties. Furthermore, the reason for the good thermal charge stability of Yuhua9830 may be due to the fact that heating might not lead to a substantial augmentation in the surface hydrophobicity of the OBs\(^{(42, 43)}\). The good thermal charge stability of OBs from various plant sources had previously been demonstrated\(^{(41)}\).

But temperature may affect the stability of the OB from another aspect. Previous studies have found that endogenous enzymes such as proteases and phospholipases are present in oil bodies, and the degradation of enzymes during storage of oil bodies also affects the stability of oil bodies\(^{(45, 46)}\). Moreover, temperature fluctuations are unavoidable in the actual application of the OB, which will lead to changes in enzyme activity and thus affect the stability of the OB. It was reported that heat treatment and storage temperatures can affect the stability of OBs, such as peanut, soybean and argan\(^{(27, 45–47)}\). It showed that heat treatment can improve the stability of oil bodies, which

### Table 5

| Temperature/°C | Yuhua23     | Yuhua27     | Yuhua9719   | Yuhua9830   | Yuhua9502   |
|---------------|-------------|-------------|-------------|-------------|-------------|
| 25            | \(-30.40 \pm 2.87\) B | \(-39.98 \pm 3.41\) A | \(-32.43 \pm 3.00\) C | \(-41.40 \pm 4.14\) A | \(-23.96 \pm 1.85\) C |
| 30            | \(-42.10 \pm 3.46\) A | \(-26.88 \pm 2.44\) B | \(-35.55 \pm 3.17\) BC | \(-43.31 \pm 3.25\) A | \(-26.38 \pm 2.66\) C |
| 35            | \(-40.21 \pm 2.73\) A | \(-25.03 \pm 2.59\) B | \(-36.09 \pm 3.68\) BC | \(-46.49 \pm 3.91\) A | \(-39.96 \pm 3.03\) B |
| 40            | \(-39.53 \pm 3.53\) A | \(-18.36 \pm 1.15\) C | \(-42.13 \pm 3.53\) A | \(-43.03 \pm 2.84\) A | \(-44.25 \pm 3.38\) B |
| 45            | \(-38.03 \pm 2.09\) A | \(-17.23 \pm 1.05\) C | \(-40.03 \pm 2.03\) AB | \(-41.03 \pm 2.24\) A | \(-46.71 \pm 3.90\) A |

Data were presented as means ± standard deviations. The data in a column marked with different capital letters were significantly different \((p < 0.01)\).
may be caused by high temperature treatment leading to decreased activity of endogenous proteases and phospholipases, and slows down the degradation of the OB protein and phospholipid membrane, thereby improving the stability of the OB
47. In this study, it was found that different varieties of peanut OBs had different trends in charge stability of oil bodies under the same temperature gradient. This may be also due to differences in the levels of endogenous enzymes, and enzymatic properties in different varieties of peanuts.

4 Conclusion

The current study demonstrated that oil bodies (OBs) with optimum stability could be successfully obtained from peanuts by aqueous extraction. The zeta potential of the extracted peanut OBs was estimated with respect to pH, ionic strength, and temperature as these conditions are frequently encountered in the food industry. The OBs extracted from five peanut varieties (Yuhua23, Yuhua27, Yuhua9719, Yuhua9830, and Yuhua9502) proved to be stable (zeta potential > 20 mV) at low salt concentrations (<10 mM) at pH 3.0 and 7.4, and the OBs from these five peanut varieties possessed the same charge (positive or negative) at pH 3.0, 7.4, and 9.0. The maximum absolute value of zeta potentials of these five peanut varieties was observed at pH 7.4 in the absence of NaCl. The zeta potentials of Yuhua27 and Yuhua9830, in particular, were as high as ~40 mV at pH 7.4 without NaCl. Moreover, the OBs isolated from the five peanut varieties, except Yuhua27, were stable (zeta potential > 20 mV) to a moderate increase in temperature in the range of 25–45°C. Yuhua9830 possessed the best thermostability between 25–45°C at pH 7.4 without NaCl, consequently, Yuhua9830 displayed the best charge stability in these conditions among the studied peanut varieties.

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