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Nano Formulated Soy Proteins for Improvement of Beef Burgers Quality

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THE QUALITY of beef burgers produced using 100% meat as a primary source is less compared to burgers produced with soy proteins. The growing field of nanotechnology presents the potential for further improvement in the cooking properties of beef burgers. This study was conducted to improve the quality characteristics of the beef burger through the usage of nano soy protein particles and nano glycinin protein. Ultrasonic cavitation was used to synthesize the nano proteins. The obtained nano proteins were characterized to evaluate zeta potential, particle size distribution, crystallinity and polydispersity index, as well as, functional properties such as water and oil holding capacity, hydrophobicity, solubility and emulsion properties. Beef burger quality was evaluated by comparing cooking loss, cooking yield, shrinkage, and pH with the negative control sample. The results showed that the shrinkage and cooking loss were decreased by increasing storage period in beef burgers prepared from the nano proteins compared with burgers prepared from soy protein isolate and glycinin. The nano glycinin protein exhibited the highest cooking yield among all proteins and was significantly higher ($P \leq 0.05$) than the negative control. After storage, percent shrinkage was significantly lower ($P \leq 0.05$) for nano soy protein and nano glycinin (18 and 17.8 %, respectively) than the negative control (30%). Plasticity for nano soy protein and nano glycinin (4.42 and 4.23, respectively) increased compared with the negative control burger samples (3.7) after 3 months. The addition of nano formulated proteins to beef burger improved the cooking quality of the meat product.

Keywords : Beef burger , Nanotechnology , Nano soy proteins , Nano Glycinin, Ultrasonication

Introduction

Nano-foods are defined as a food which processed, packaged or produced using nanotechnology techniques. Nanotechnology can revolutionize food processing by creating nano nutrient delivery systems, increase the nutritional values and generate novel products through encapsulation of bioactive compounds. Nanotechnology has been used to improve processes in food manufacturing, such as food packaging, processing and storage.

Nanoformulation can enhance the bioavailability and absorption of nano- nutraceuticals and supplements, improve product taste, flavor, increase shelf life, stability, and lastly improve the texture of food products [1, 55].

Soy proteins are classified according to their sedimentation velocity and are separated into four protein fractions; 2S, 7S (*β*-conglycinin), 11S (Glycinin) and 15S. Glycinin and *β*-conglycinin proteins are the major predominant fractions

and represent about 65- 80% of the total soy proteins [2]. *β*-conglycinin (7S) is a trimer poly peptide consisting of subunits α', α and *β* linked by non-covalent bonds [3-5]. Glycinin (11S) is the second major component in soy proteins and it is a hexameric protein consisting of A and B polypeptides linked by disulfide bonds [3].

Many approaches have been used to produce nano form of soy bean proteins such as homogenization, sonication etc. In sonication approach, high intensity ultrasound (HUS) is used in liquid systems to create the micro streaming and cavitation phenomenon [6, 7]. The cavitation bubbles are formed during the sonication treatment, grow and collapse sharply over a few cycles, resulting in high pressures and high temperatures. High shear energy waves and turbulence was created in the cavitation zone as reported by Hu et al. [8]. Functional properties of soy protein isolate are correlated with protein surface and the structure parameters, *i.e.* porosity, total pore volume, pore radius and particle distribution [9-12].

Meat and meat products are the most important protein sources in the human diet. Beef burger is the most popular meat product consumed by millions of people from all over the world [13, 14]. Soy proteins are used in meat products for their important functional properties (*e.g.* binding properties for water and fats, cooking yield, product texture, and gelling capacities) [15].

The present study aimed to use a well prepared and characterized natural nano sized soy protein isolate (NSPI) and glycinin to improve the cooking attributes of beef burgers. The prepared nano formulations were characterize by TEM, FTIR, Zeta potential, XRD and particle size distribution, as well as functional properties (protein solubility, water holding capacity, oil holding capacity and emulsifier properties) were also evaluated. Four beef burger products were prepared by adding soy protein isolate (SPI), nano soy protein isolate (NSPI), glycinin (GLY) and nano glycinin (NGLY) proteins. The samples were tested to determine the cooking parameters at zero time and after 3 months freeze storage.

Material and Methods

Soy bean seeds (Varity: Giza, 72) were obtained from the Experimental Station, Agricultural Research Centre, Giza, Egypt. All used chemicals were of analytical grade,

Sodium chloride, sodium hydroxide, sodium bisulfate and hydrochloric acid were purchased from Sigma-Aldrich (St., louis, MO, USA). Soy protein isolates (SPI) and glycinin (GLY) were extracted from soy bean seeds and the obtained precipitates were lyophilized according to Nagano et al. [16]. Beef meat was purchased from a local butcher shop at Giza Governorate, Egypt. Other ingredients: spices and salt were obtained from the local market at Giza.

Preparation of nano soy protein (NSPI) and nano glycinin (NGLY)

Batches of 3 gm soy protein isolate or glycinin were dispersed in 100 ml distilled water and stirred at room temperature for 2 hour. Samples were treated with High Intensity Ultrasonic waves (DAIGGER, Model GEX 750, USA) using a frequency of 20 kHz and intensity of 400 W/cm^2 for 40 min according to Hu et al. [8]. During sonication, the beaker containing the protein dispersion was placed in an ice bath to maintain the temperature under 50° C. All samples were lyophilized after treatment in a freeze drier (CHRIST LSC plus, ALPA 1- 4, Germany) and stored at 4°C until the following experiments.

Physico-chemical characterization of NSPI and NGLY

Determination of UV absorption of glycinin and soy protein isolate was carried out by UV-Vis Double Beam/ UVD 3500 nm (Labomed Inc. California USA) at wave length range 200-700 nm after the samples had been dissolved in distilled water. Fourier-transform infrared spectroscopy **(**FT-IR) analysis of NSPI and NGLY was carried out by using FT-IR spectroscopy (FT-IR 6100 Jasco, Japan). Samples were prepared using Potassium Bromide disks. FT-IR spectral resolution was 4 cm^{-1} and wave number ranged from 400 – 4000 cm^{-1}. Crystallity of nano soy protein isolate and nano glycinin samples were evaluated using XRD techique (PANalytical X'Pert, PRO X ray machine, Netherland) according to Jin et al. [17].

Soy protein isolate (NSPI) and glycinin nanoparticles (NGLY) were imaged by a high resolution transmission electron microscope imaging (HR-TEM, Tecnai G20, FEI, Netherland) operating at 200 kV using lanthanum hexaboride (LaB$_6$) electron source gun according to Jin et al. [17]. Samples were prepared by adding one drop of NSPI and NGLY suspension on TEM grids coated with carbon film, blotted on filter paper for 1 min to totally absorb suspensions on grids and dried at room temperature.

Zeta potential, polydispersity (PDI) and Particle size Distribution of NSPI and NGLY were determined at room temperature using Zetasizer Nano-ZS90 (Malvern Instruments Ltd., UK).The samples were suspended in distilled water for analysis according to Hu et al [8]**.** The polydispersity is a measure of the size distribution of the nanoparticles.

Functional properties for SPI, NSPI, GLY and NGLY samples
   Protein solubility
   Dispersion of 20 mg of proteins sample in 20 ml distilled water at different pHs (3, 4.5 , 7, 9 and 11), were magnetically stirred for 30 min and centrifuged at 10 000 Xg for 10 min, protein solubility was calculated according to Wu et al [18] as follows:

Solubility (%) = (protein content in supernatant) / (total protein content in sample) x 100.

   The protein content (total) and the supernatant was determined by micro kjeldahl method using factor 6.25.

Water Holding Capacity (WHC)
   According to Heywood et al. and Traynham et al. [19, 20] , water holding capacity was determined with slight modification. A volume of 10 ml distilled water was added to 30 ml plastic centrifuge tubes (pre-weighed) containing 2 gm of SPI, NSPI, GLY and NGLY powders. After mixing, the mixture was left at 25 ±1°C for 30 min, after which it was centrifuged at 4000 Xg for 30 min. The supernatants were decanted and the mass of the tube after centrifugation was measured.

   WHC g water / g powder was calculated as follows:

W*HC* (g / g powder) = total water mass / dry

mater mass
Hydrophobicity
   The SPI, NSPI, GLY and NGLY samples were individually pressed under pressure to achieve a cubic shape. Then a water droplet is deposited by a syringe which is positioned above the sample surface, a high resolution camera captures the image and determine the contact angle which is measured via the sessile droplet technique.

Oil holding capacity
   Oil holding capacity (OHC) was determined according to Chakraborty [21] with some

modifications: 10 ml of sunflower oil (density = 0.898 g /ml) were placed in 30 ml plastic centrifuge tube (pre weighed) contained 1 gm SPI, NSPI, GLY and NGLY in each tube. The samples were mixed well at maximum speed in a Vortex mixer, after which all samples were left at 25 ±1°C for 30 min. The samples were then centrifuged at 4000 Xg, the supernatant was decanted and the mass of sample after centrifugation was determined. OHC g oil / g dry powder was calculated as follows:

$$OHC \text{ (g oil /g powder)} = (m_{oiled} - m_d) / m_d$$

Where m_d and m_{oiled} are the mass of dry powder material and the mass of sample including held oil, respectively.

Emulsifying properties
   Six ml of 1% protein suspension at pH 7 was add to 2 ml corn oil then homogenized for 1 min, then 50 µl of the emulsion from the bottom of tube at 0 and 10 min were pipetted diluted with 5 ml of 0.1% SDS. Absorption was observed at 500 nm using double beam spectrophotometer according to Pearce and Kinsella [22]**.** The absorbency measured immediately (A_0) and at 10 min (A_{10}) after emulsion formation were used to calculate the emulsifying activity index (EAI) and the emulsion stability index (ESI) as follows:
EAI = 2T (A_0 × dilution factor / C × Φ × 10,000)………… (cm²/g)

Where T = 2.303; A_0 = absorbance measured immediately after emulsion formation; dilution factor = 100, C = weight of protein/unit volume (g/ml) of aqueous phase before emulsion formation; and Φ = oil volume fraction of the emulsion.

$$ESI = A_0 × \Delta t / \Delta A \text{ …………… (min)}$$
Where Δt = 10 min and $\Delta A = A_0 - A_{10}$.

Preparation of beef burger
   Preparation of beef was carried out in agreement with Egyptian standard specification for burger [23] with some modification as follows:

   Minced meat 65%, fat 20%, black pepper 0.3%, salt 1.8% , red pepper water 10% and SPI, NSPI , GlY and NGLY 5%. All ingredients were handily mixed and formed into 50 g beef burgers by using manual burger press machine, packed in foam plates and frozen stored at -18 ºC for 3 months.

pH Measurement
   Beef burger sample (1 g) was homogenized

with 10 ml deionized water for 1min. pH was measured at room temperature using a digital pH meter (Cyber Scan pH 500, USA).

Water holding capacity and plasticity

Water holding capacity (WHC) and plasticity of samples were measured using the method of Wierbiciki and Deatherage [24] as follows: 0.3 g burger samples were put on an ashless filter paper (Whatman No.41) and pressed for 10 min using 1 kg weight. Two zones were formed on the filter paper, and by digital planimeter (Koizum Placom, KP-90) their surface areas were measured. The outer zone resulted from water separated from the pressed beef tissues, which is an indicator to the water holding capacity and the internal zone was due to the pressing only is an indicator to the plasticity. The water holding capacity was calculated by different between the area of the internal zone from that of the outer zone. Data were presented as cm^2 as described by Russo et al. [25].

Cooking procedure and cooking measurements

Samples were cooked in oven, the oven was preheated to 185°C for 10 min. All cooking measurements were done on three replicates of each treatment. The cooking yield was determined as reported by Naveena et al. [26] as follows;

Shrinkage measurements (%)

Raw and cooked samples were measured for diameter and thickness of burger as described by Barry [27]. The reduction in diameter and width for burger samples was determined with Vernier Caliper at two and three positions to obtain the mean values.

Dimensional shrinkage was calculated using the following equation as reported by Murphy et al. [28].

Sensory evaluation

Odor, juiciness, flavour, tenderness, appearance and overall acceptability of beef burger samples were assessed by 15 members of Food science and technology department, National Research Centre (with past experience in burger processing and evaluation) to evaluate their sensory characteristics. Sensory hedonic scheme, ranged from 0 (very bad) to 9 (very good) as described by A.M.S.A. [29].

Statistical analysis

All experimental data were analyzed by one - way analysis of variance (ANOVA), using statistical analysis system (SPSS). All samples were compared using Waller-Duncan multiple range tests at the 0.05 level of significance [54].

$$\text{Cooking yield (\%): } = (\text{Cooked sample weight}) / (\text{Uncooked sample weight}) \times 100$$

$$\text{Cooking loss (\%):} = \frac{(\text{Uncooked sample weight}) - (\text{Cooked sample weight})}{(\text{Uncooked sample weight})} \times 100$$

Results and Discussion

Morphology of nano soy protein isolate and nano glycinin

Transmission electron microscope (TEM) images of the SPI and NSPI particles were examined. The morphologies of the nano scale distribution of tested materials (SPI) are shown in Fig. 1 a, b. The particles were near spherical shapes with some aggregates and the size for the soy protein isolate particles ranged from 170 nm to 330 nm. The nano soy protein size ranged from 15 nm to 30 nm. These results were in agreement with those reported by Zisu et al [30].

Figures 1 c and 1d show the TEM images of GLY particles and NGLY aggregates. The particle size of glycinin was in the range of 6.9 to 16.6 nm, while that of nano glycinin was in the range of 41 nm to 64.7 nm this may be due to aggregation of NGLY particles by ultrasonication treatment.. These photographs indicated that the NSPI and NGLY particles were successfully formed. These results agreed with several studies which examined the effect of sonication on size of proteins [3, 31, 32].

$$\text{Shrinkage (\%)} = [(\text{Raw thickness - Cooked thickness}) + (\text{Raw diameter - Cooked diameter}) / (\text{Raw thickness + Raw diameter})] \times 100$$

Ultra Violet –Vis Spectroscopy

The UV spectrum of SPI (control), NSPI, GLY and NGLY samples are shown in Figs. 2 a, b, c, and d. The intensity of UV absorbance for SPI is more than NSPI protein.

The main peak of the soy protein isolate was located at 208 nm as show in Fig. 1 a, while the main peak of the nano soy protein sample was shifted and was located at 202 nm (Fig. 1b).

Moreover, the absorption intensity of soy protein isolate was 3.649 while that for nano soy protein was 3.187. Figure 2 c, d shows the UV-Vis spectra for GLY and NGLY samples, respectively. The absorption peaks for GLY and NGLY samples appeared at 258 and 257 nm, respectively. There is a left shift in the absorption peak in the case of nano glycinin sample compared to that of glycinin. A small peak was also found at 208 nm.

XRD measurement

X-ray diffraction technique is an analytical technique that can produce information about crystallographic structure, particle size and chemical composition of samples based on measurements of the intensity of an X-ray beam after it hits a sample. The crystallographic structure of SPI and NSPI particles are shown in Fig. 3. Glycinin and nano glycinin samples were also examined by XRD and the obtained graphs are shown in Fig. 4.
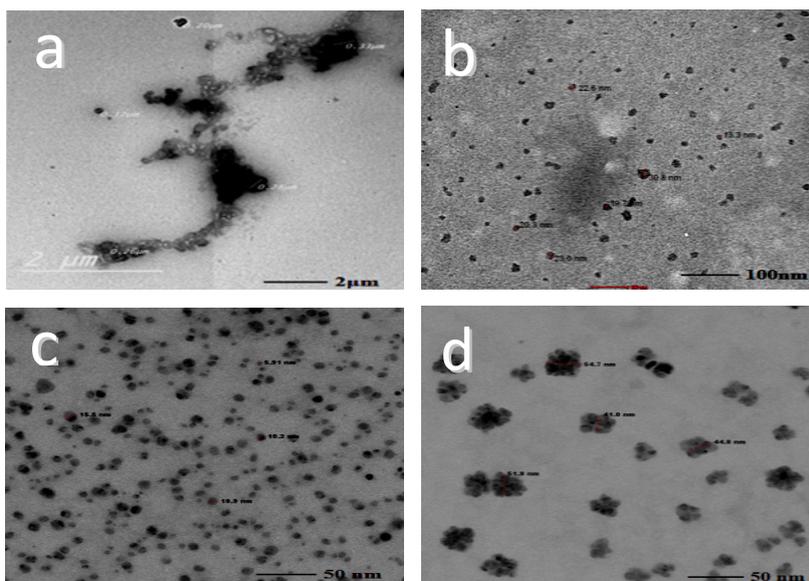

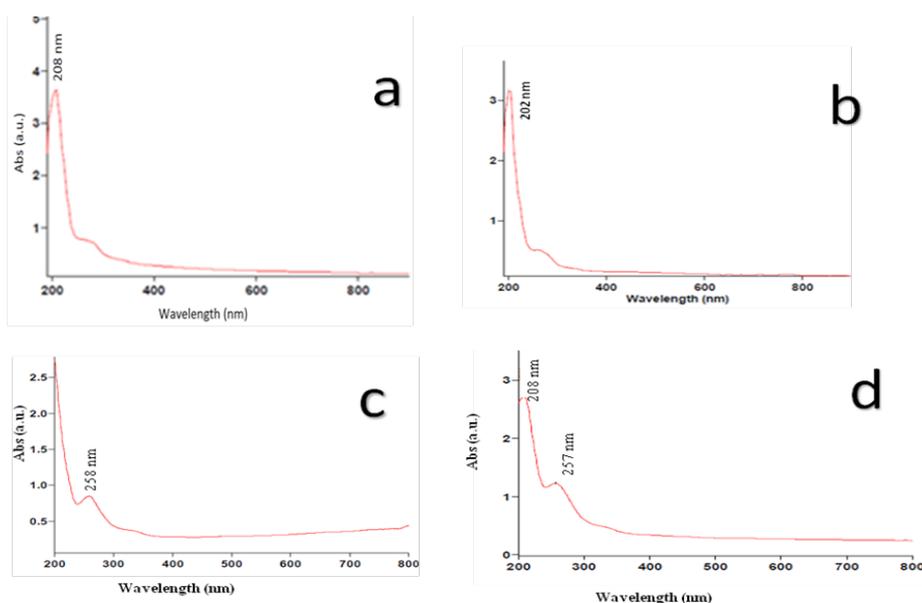**Fig. 1. Transmission electron microscopic images. (a) SPI, (b) NSPI, (c) GLY and (d) NGLY.**

Fig. 2. UV spectrum of SPI (a), NSPI (b), GLY (c) and NGLY (d).
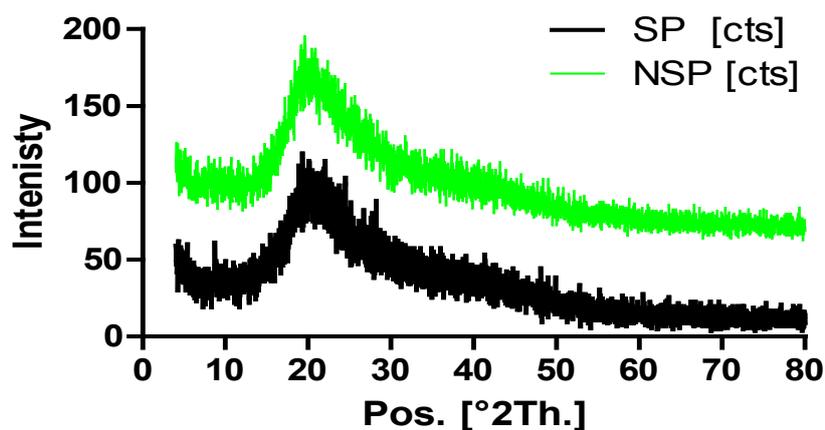
Fig. 3 . XRD spectra of SPI and NSPI.
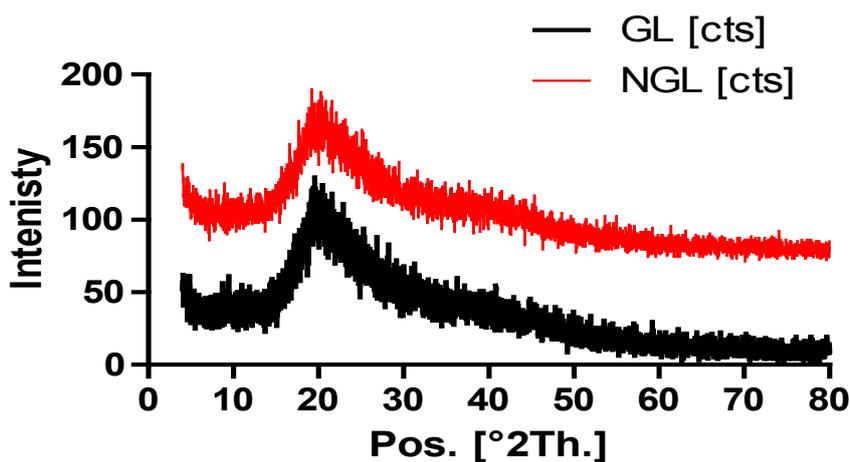
Fig. 4 . XRD spectra of GLY and NGLY.

The SPI and NSPI particles showed a high crystalline peak at 2 θ value of about 19° - 20° and a small peak at 2 θ value of about 8° - 9° Figure 3 shows the XRD for SPI and NSPI samples. As seen, there is a shift in the angle and intensity of both samples in the X- ray pattern (2θ = 20.8° for SPI sample and 19.8° for NSPI sample, intensity =100 %). This may be due to cracking in soy protein particles and increasing in the space between particles. These results were confirmed by Zhao et al. [33] who mentioned that 2θ for

soy protein (β-sheet and α-helix structure) was around 10° and 20°. These results also indicated that there were no change in the protein, but there was a slight decrease in β- sheet structure and low crystallite structure.

The XRD pattern for GLY and NGLY samples shown that $2\theta = 19.7$ ° and 19.9°, respectively, as shown in Fig. 4. Results indicated that crystallity for nano glycinin increased slightly after ultrasonic treatment. The change in crystallinity may be due to the aggregation of glycinin particles.

Particle size distribution, polydispersity and zeta potential

The measurements of polydispersity index and zeta potential of the (SPI, NSPI, GLY and NGLY) particles in potential nano dispersions were summarized in Table 1.The polydispersity index (PDI) was 0.462 and 0.414 for SPI and NSPI particles, respectively, which revealed that nanoparticles were uniform distributed and the same trend was observed for NGLY particle. Where its PDI - value was 0.567.

Zeta potential (ζ) is the potential difference between the dispersion medium and the stationary layer of fluid. It is an indicator for the stability of the colloid in the protein emulsions through the determination of the charges of the proteins [34]. The increase in zeta potential value (ζ) resulted in higher stability of NSPI colloidal compared with SPI. As shown in Table 2. Zeta potential values of GLY and NGLY samples were similar and the difference was negligible (0.4). This means that there is no difference in the colloidal stability of GLY and NGLY samples as shown in Table 2.

Zeta sizer Zs 90 Malvern (Instruments Ltd., UK) instrument was used to measure the particle size distribution as shown in Figures 5 a, and b The determined particle size values for SPI and NSPI samples were 123.9 and 63.09 nm, respectively, which agree with TEM results. On other side, NGLY sample showed particle size (around 223 nm) than GLY sample (around 85). This is probably due to the aggregation of the glycinin particles as a result of ultrasonic treatment as suggested by Fukushima and Hu et al. [3, 8].

Fourier transforms infrared (FTIR) measurements

Figure 6 Shows the FTIR- spectrogram of soy protein isolate (SPI) and nano soy protein isolate (NSPI). The peaks ranged between 2925 cm^{-1} and 2928 cm^{-1} as well as at 2857 cm^{-1} are characteristic peaks for alkanes (C-H) group and in the same time, this peak is a characteristic point between the functional groups with frequency higher than 3000 cm^{-1} and those lower than 3000 cm^{-1}. The intensity of these two (C-H) peaks were slightly higher for NSPI sample which may be due to the destruction of the macro SPI molecules to smaller particles through sonication treatment.

As seen in the spectrogram, the small peak at 3010 cm^{-1} is a characteristic peak indicator for the presence of alkenes (C=CH). This component is present in small amount in SPI and has disappeared (through destruction) in the spectrogram of the NSPI sample. The peak at 3410 cm^{-1} is a characteristic peak for either O-H (alcohol) or N-H (amines group) and for SPI samples, it is most probably related to free amine groups, which are able to form hydrogen bond with carbonyl group of the protein. As seen, the intensity of this peak was reduced from 74.85 % in SPI samples to 71.71 % for NSPI sample.

TABLE 1. Polydispersity index and zeta potential of SPI, NSPI, GLY and NGLY particles.

Samples	Polydispersity index	Zeta potential (mV)
SPI	0.462	-27.3
NSPI	0.414	-34.3
GLY	0.79	-25.1
NGLY	0.567	-25.5

TABLE 2. Water holding capacity, oil holding capacity and contact angle for SPI, NSPI, GLY and NGLY samples.

Samples	Functional properties		
	Water holding capacity (g/g)*	Oil holding capacity (g/g)*	Contact angle (º)
SPI	2.7 ± 0.46 [c]	1.76 ± 0.46 [b]	75.2
NSPI	5.79 ± 0.30 [a]	3.42± 0.30 [a]	87.9
GLY	2.28 ± 0.83 [c]	1.83 ± 0.83 [b]	18.5
NGLY	4.03± 0.4 [b]	4.63 ± 0.46 [a]	72.3

*Means with different letters in the same column are significantly different ($P \leq 0.05$), ± stander error
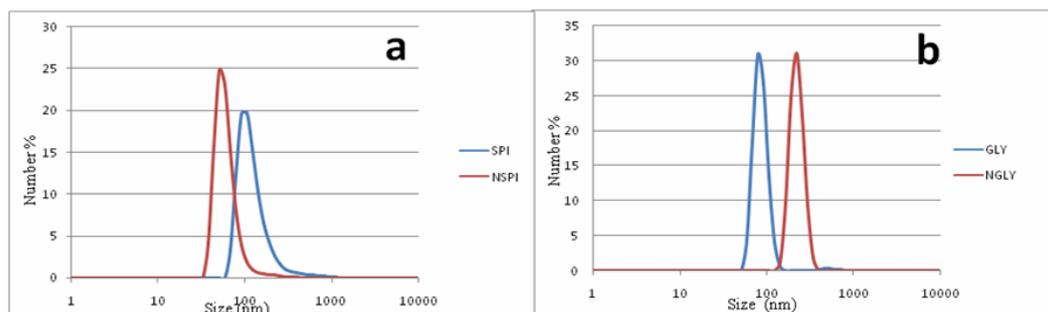
Fig. 5. Particle size distribution of SPI and NSPI (a), GLY and NGLY (b).
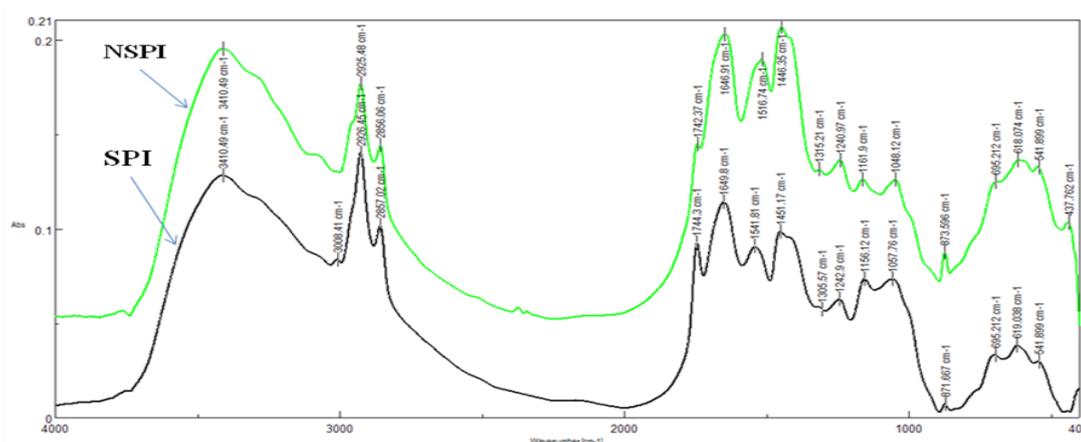

Fig. 6. FTIR spectrogram for soy protein isolate and nano soy protein isolate.

Most of the peaks present on the right side of the FTIR spectrogram are characteristic of molecular bonds (amide bonds) in the peptide structure of SPI protein. Peaks at 1744 cm^{-1} and 1649 cm^{-1} are characteristic of the amide I type bond (C=O) of the protein. These two peaks belong to the α-helix confirmation and *β*-sheets confirmation of the proteins and arise from the stretch of the C=O group in the peptide molecule. The intensity of these peaks are 80.9 and 77.01 % in the SPI molecule and 80.5, 70.44 % in the NSPI molecule. From these results it appears that one of the two forms of the peptide configuration was reduced during the sonication treatment.

In addition, the peaks at 1541 and 1451 cm^{-1} belong to the amide II bond which mainly represent the deformation vibration of the N-H group associated with stretching vibration of C=O bond in the peptide molecule. As seen, the intensity of this amide bond was reduced from 81.2 and 79.8 % in SPI molecule to 72.6 and 69.7 % in NSPI molecule, indicating that sonication induces a reduction in the N-H bond of the SPI molecule. The change in intensity in the amide II bands reflects the conformational changes in the tertiary structure of the protein [35].

The peak at 1240 – 1242 cm^{-1} is a characteristic peak for amid III bond which is a very complex bond in proteins arising from the deformation of N-H and C-N stretching (C-N stretching vibration matching to NH$_3$ group of the amino acid). The intensity of this peak was also reduced from 86.6 in SPI molecule to 82 % in NSPI molecule. A new peak appeared at 1314 cm^{-1} in the NSPI molecule with an intensity of 83% and it was not found in the raw SPI sample. It is assumed that this peak also belongs to the amide III bond in the NSPI molecule.

There was no remarkable difference in the intensity peaks of SPI and NSPI between wave lengths 872-1314 cm^{-1}. At wave number 700 cm^{-1}, there is a peak with intensity 92 % which was found in the SPI sample and not found in the NSPI sample. This peak is related to the deformation vibration of the C-H bond and it agrees with the results of alkans intensity found at 2988 and 2857 cm^{-1} in the present work. The peak at 544-547 cm^{-1} is characteristic of the absorption of S-H groups in the protein. The intensity of this peak was slightly changed from 83% to 82 % in NSPI samples. This change in SPI intensity and shift in position could be a result of the formation of S-S bonds as indicates by Hu et al. [8]**,** due to the reactions with free radicals produced during sonication. From these results it is obviously clear that sonication induced changes in the types and intensities of the amide bonds in the protein molecule of these two samples. These results agree with those reported by Susi and byler [36] who mentioned that shifts to lower frequencies and band broadening are indicators of the loosening of the protein conformational structures.

Figure 7 shows the FT-IR spectrogram of glycinin (GLY) and nano glycinin (NGLY). As seen, no changes have been occurred in the positions and intensity of free amine groups (wave number of 3417-3418 cm^{-1}), where it is recorded an intensity of 69 %. However, the intensity of the alkanes group (C-H) at wave number of 2925 and 2858 cm^{-1} was increased to 74.7 and 80 % in NGLY sample compared with 71.2 and 78.2 % for

GLY sample. This probably means that some new C-H groups are accumulated in NGLY through the aggregation process during the production of NGLY. On the other side, no changes have appeared in the position and intensity of amide I (C=O) of the peptide molecule (wave number 1741 and 1644 cm^{-1}), which indicate that no changes in the C=O group have been appeared in GLY or NGLY samples. The location of the amide II bands (vibration of the N-H group) is observed between wave length 1403.9 and 1537 cm^{-1}. The intensity of amide II of NGLY sample was higher than that of the GLY sample which indicates that the NGLY molecule contain more N-H groups than that of GLY molecule, which could be a result of the aggregation process. The spectrogram in Fig. 7 also show moderate increase in the vibration bands of carbonyl groups (C=O) (1442 to 1451 cm^{-1}) and an increase in the vibration bonds of C-C bond, which could be referred to the aggregation happened in NGLY sample.

The spectrogram in Fig. 7 shows also no remarkable changes in the location of amide III bands between GLY and NGLY samples. From this result it is clear that the intensity of the spectrogram of the NGLY sample, in general, was higher than that GLY sample which could be referred to the aggregating reaction during the production of NGLY, which agree also with the result of particle size and zeta- potential of both compound given in the present work . Our results are in agreement with those of Ravichandran and Wang et al. [7, 37].

Fig. 7. FTIR spectrogram for glycinin and nano glycinin.

Functional properties for SPI, NSPI, GLY and NGLY samples

Water Holding Capacity (WHC)

There was a significant difference ($P \leq 0.05$) between WHC for NSPI and SPI. The WHC for NSPI was 5.79 ± 0.30 g/g and SPI was 2.7 ± 0.46 g/g as shown in Table 2. Our results agree with those of Hu et al and Ouyang et al [38,8], who found that the water holding capacity for the molecule in nanoscale was increased compared with the large molecule. This may be due to reduction in size (hence, increased surface area) which occur during ultrasonic process to production of nano soy proteins.

As shown in Table 2, water holding capacity for nano glycinin sample was increased from 2.28 ± 0.83 g/g to 4.03 ± 0.4 g/g for glycinin sample after ultrasonic treatments at 400 W for 40 min. It is due to the aggregation process during production of the nano glycinin sample.

Hydrophobicity surface is an index of the hydrophobic groups on the surface contacted with the polar aqueous and is closely related to its functional properties [3]. Hydrophobicity of SPI, NSPI, GLY and NGLY samples were determined with contact angle methods and the results are illustrated in Table 2. The contact angles of SPI and NSPI samples were increased from 75.2° to 87.9°, respectively, while the contact angle for NGLY sample was higher (72.3°) than the contact angle of GLY sample (18.5°). The contact angle was increased during ultrasonic treatment. This increase in hydrophobicity may be due to the cavitation phenomenon caused by ultrasonic and destruction of large particle of SPI sample for production of NSPI sample, which displayed some of the submerged hydrophobic regions of the SPI sample in the interior of the molecules to the surface. These results are in agreement with results of Hu et al and Zhao et al. [8, 33], who showed that ultrasonication, could cause an increase in surface hydrophobicity for proteins. Oil holding capacity (OHC) for SPI, NSPI, GLY and NGLY samples are shown in Table 2. As seen, SPI sample recorded the lowest OHC (1.76 g/g) at ($P \leq 0.05$) compared with NSPI, GLY and NGLY samples. The highest OHC (4.63 g/g) was for nano glycinin sample. The increase in OHC in nano samples after HUS treatment may be a result of changes in size and hydrophobicity of the protein. This result agree with Acosta-Domnguez et al. [39], who observed that the oil absorption capacity of NSPI sample was higher than that of

SPI sample, which may be due to the presence of nanocavities that increase the energy in the surface of NSPI sample.

Protein solubility

Figure 8 a and b shows that the solubility of the protein was higher in SPI than NSPI samples and increased from 42.1, 24.7, 66.5, 68.4 and 74% (for SPI sample) to 43.6, 25.1, 76.5, 77.8 and 83.1% (for NSPI sample), respectively, as show in Fig. 8 a, b. The increase in solubility may be due to formation of soluble protein aggregates from insoluble protein aggregates, and the conformational changed during ultrasonic treatment [40]. The sonication treatment can reduce the particle size of NSPI sample and increase the interactions between water and protein resulting an increase in solubility of the protein [3, 35, and 41]. The decrease in solubility at 4.5 for soy protein isolate samples occurred as the isoelectric point of soy proteins (between pH 4 and 5) and the lowest solubility was at pH 5 to 6 for glycinin [42].

Figure 8 c and d shows the solubility of glycinin and nano glycinin. The solubility for NGLY sample was slightly increased at pHs (5, 7, 9 and 11), while the solubility of NGLY sample at pH (3) decreased from 39.6 to 34 % compared with GLY sample. These results were in agreement with those mentioned by Jambrak et al, Hu et al, Chen et al, Tang et al and Karki et al [2, 32, 35, 40, and 41].

Emulsion Stability Index

Emulsifying Activity Index (EAI) of SPI, NSPI, GLY and NGLY samples were presented in Fig. 9. As shown in Fig. 9, a, EAI was higher than for NGLY sample at all pHs (4.5, 7, 9 and 11) except at pH 3. These results are confirmed with those of Jambrak et al. [2], who mentioned that high intensity ultrasound (HUS) increased EAI of SPI. Chen et al 2011 [35] reported that HUS caused a reduction in the drop size in emulsion, which are due to partial denaturation of the quaternary structure of the protein and structure changes that could be cause an increase in EAI and the adsorption of protein.

The EAI of SPI and NSPI at pH 3 was the lowest (142.78 cm²/g) for SPI sample and increased after ultrasonication treatment to 191.53 m²/g for NSPI sample. The highest emulsion capacity was at pH 11 after ultrasonication (214.56 cm²/g) as shown in Fig. 9 c.
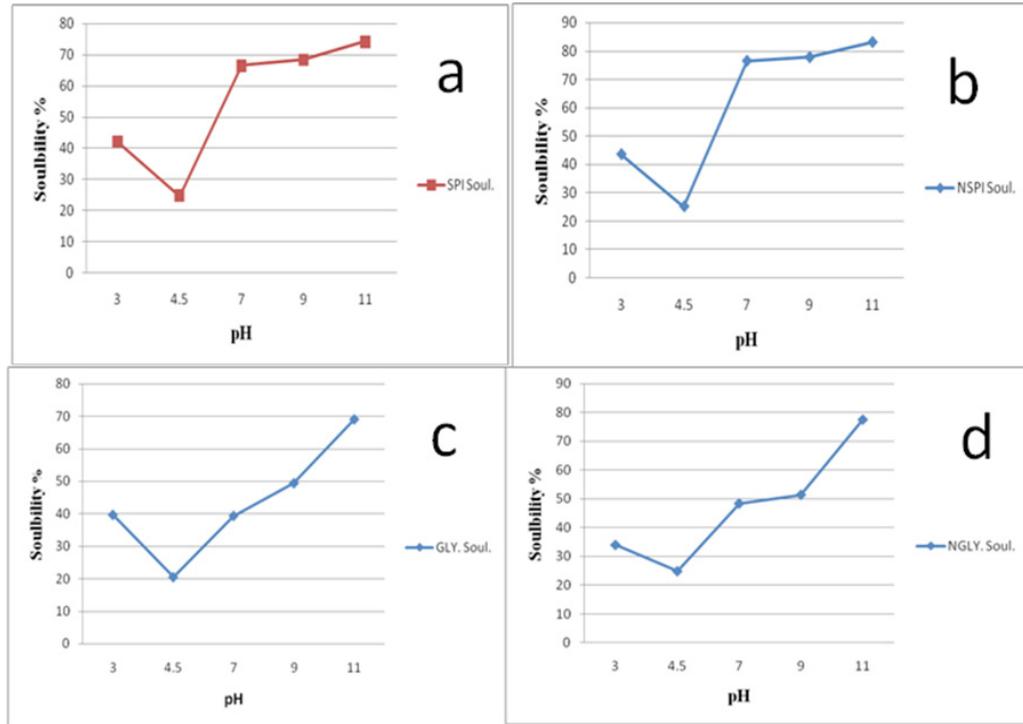
Fig. 8. Protein solubility of SPI (a) NSPI (b), GLY (c) and NGLY (d) at different pHs.
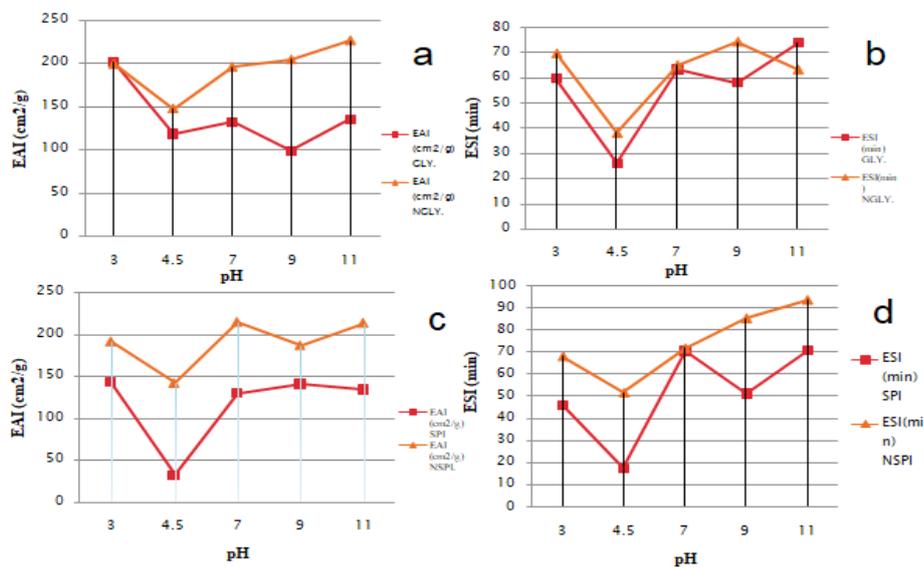

Fig. 9. EAI of GLY and NGLY (a), SPI and NSPI (c) and ESI of GLY and NGLY (b), SPI and NSPI (d) at different pHs.

Emulsion Stability Index (ESI) of SPI and NSPI samples are presented in Fig. 9 d. The Emulsion Stability Index of NSPI sample was higher at pHs (3, 4.5, 9 and 11) than SPI sample. However, at pH 7, the ESI was the same value for SPI and NSPI samples. These results were in agreement with Jambrak et al [2], who assumed that ultrasound treatment generated the soluble aggregates that lead to a better potential adsorption at the oil–water interface. Emulsion Stability Index of GLY and NGLY samples are presented in Fig. 9 b. There was a significant difference between GLY and NGLY samples ($P \leq 0.05$). The emulsion stability index was high for NGLY sample at pH 9 compared to GLY sample. The increased emulsion stability index (ESI) may be due to the effect of the cavitation phenomena that occurred during ultrasound treatment.

pH and shrinkage of the beef burgers
The pH values and shrinkage of the beef burgers at time zero and after 3 months are presented in Table 3. The pH of the burger samples is as follows: 5.94, 6.53, 6.56, 6.63 and 6.74 for the negative control, SPI, NSPI, GLY and NGLY, respectively. The NGLY recorded the higher pH compared with the negative control, SPI, NSPI, GLY. The results in Table 3 show a decrease in pH at the end of storage period, the negative control decreased slightly from (5.9 to 5.8), SPI (6.5 to 6.4) GLY (6.6 to 6.5). There was no change in pH for NSPI and NGLY burgers. The decrease in pH after the end of storage period may be due to breakdown of glycogen and accumulation of lactic acid during frozen period [43]. These results are in agreement with Leygonie et al [44] who suggested that the decrease in pH of frozen/

thawed meat could be related to the denaturation of proteins, and/or an increase in hydrogen ions concentration due to water loss.

As shown in Table 3, ANOVA detected significant differences among shrinkage measurements for beef burger samples ($P \leq 0.05$). The lowest shrinkage percent at zero time was for NSPI and NGLY burgers (16.82± 0.21and 17.53± 0.45), respectively. Also, the shrinkage percent was increased during freeze storage period for all beef burger samples. These results did not agree with the findings of Ali [46], who concluded that no significant differences in shrinkage percentage were found in burger samples. It was however, noted that burger shrinkage was significantly affected after freeze storage.

Changes in plasticity
The following table contains the mean plasticity for burger samples that was processed with SPI, NSPI, GLY and NGLY. The plasticity at time zero was significantly ($P \leq 0.05$) higher in NSPI and NGLY (4.21 and 4.33 cm²) than the other burger samples, there was a slightly increase in the plasticity of all burger samples after the end of storage period (3 months). These results disagreed with those found by Zaki and Ali [45, 46] who found that the progress of frozen storage of beef burger at -20 ºC for 3 months was led to a decrease in plasticity.

The ability to bind water in a soy-beef product is one of the important quality attributes of soy protein- meat product. The SPI, NSPI, GLY and NGLY beef burger decreased significantly ($P \leq 0.05$) compared with the negative control sample. While there was no significant difference

TABLE 3. pH and Shrinkage for beef burger supplemented with SPI, NSPI, GlY and NGLY proteins at zero time and after 3 months.

Beef samples	pH zero time*	pH 3 months*	Shrinkage % Zero time*	Shrinkage % 3 months*
Negative control	5.94 ± 0.04 [b]	5.83 ± 0.27[b]	27.042± 0.31[a]	30.03± 0.15[a]
SPI	6.53 ± 0.51 [a]	6.48 ± 0.07[a]	24.26± 0.17[b]	25.63± 0.24[b]
NSPI	6.56 ± 0.25 [a]	6.51 ± 0.62[a]	16.82± 0.21[c]	18.08± 0.18[c]
GLY	6.63 ± 0.14 [a]	6.58 ± 0.34[a]	23.88± 0.62[b]	24.89± 0.21[b]
NGLY	6.74 ± 0.32 [a]	6.73 ± 0.51 [a]	17.53± 0.45[c]	17.84± 0.34[c]

- Means with different letters in the same column are significantly different ($P \leq 0.05$), ± standard error

between NSPI and GLY although there was still overall improvement in WHC compared with the negative control. Water holding capacity of burger produced by adding NGLY showed the lowest WHC 1.41± 0.07 cm² at ($P \leq 0.05$) than burgers of (negative control, SPI, NSPI and GLY. Also there was no significant difference between GLY and NSPI burger (1.62± 0.06 and 1.69± 0.06 cm²) as shown in Table 4.

These results agreed with Hutton and Campbell [47] whose finding reported that the functional properties such as emulsification, water holding capacity and stability of the processed foods improved as a result of adding soy proteins. Water holding capacity for SPI, NSPI, GLY and NGLY burger samples significantly ($P \leq 0.05$) decreased compared with the negative control. However, there was no significant different between NSPI and GLY burger at zero time.

Water holding capacity of burger produced by add NGLY burger shows the lowest WHC (1.41 ± 0.07 cm²) at ($P < 0.05$) than the negative control, SPI, NSPI and GLY burgers at time zero, but GLY burger showed the lowest WHC after the end of storage period (3 months). Water holding capacity was significantly different ($P \leq 0.05$) between all soy protein samples and the negative control after freeze storage. The highest WHC reported was (2.88 ± 0.02 cm²) at the end of storage period. These results are in agreement with Ali [46] who found the uncooked beef burger showed an increasing in area of released water, indicating a decrease in WHC during freeze storage at -20 °C. Soy proteins can improve water and fat binding and hence aid in emulsion stabilization in meat products, such as frankfurters.

Cooking loss and cooking yield
Figure 10 a, b shows the cooking loss and cooking yield of the negative control, SPI, NSPI,

TABLE 4. Effect of nano proteins on WHC and Plasticity for burger produced by add SPI, NSPI, GlY and NGLY at zero time and after 3 months.

Sample	WHC (cm²) Zero time*	WHC (cm²) 3 months*	Plasticity (cm²) Zero time *	Plasticity (cm²) 3 months*
Negative control	2.5± 0.1[a]	2.883± 0.02[a]	3.783± 0.02[b]	3.783± 0.02[b]
SPI	1.983± 0.02[b]	2.266± 0.05[b]	3.916± 0.07[a]	4.016± 0.25[a]
NSPI	1.69± 0.06[c]	2.18± 0.16[b]	4.216± 0.10[a]	4.236± 0.07[a]
GLY	1.62± 0.06[c]	1.833± 0.05[b]	3.916± 0.02[a]	3.936± 0.02[a]
NGLY	1.41± 0.07[d]	2.033± 0.20[b]	4. 33± 0.05[a]	4.423± 0.05[a]

- Means with different letters in the same column are significantly different ($P \leq 0.05$), ± standard error
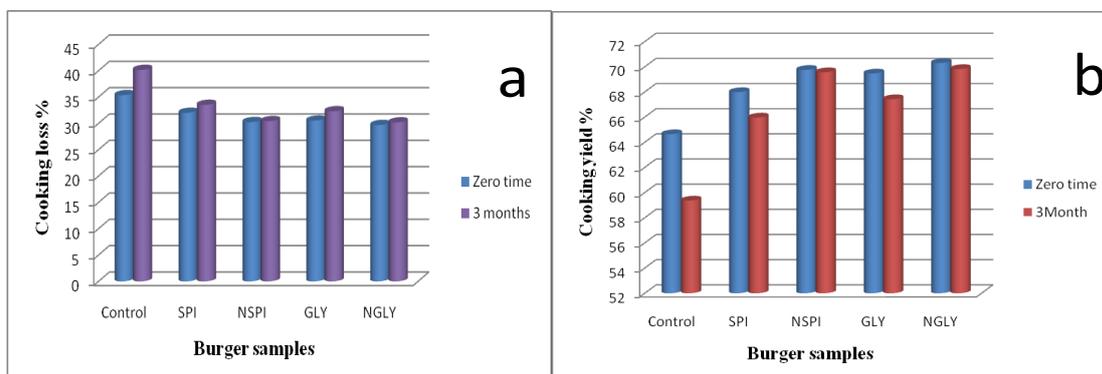

Fig. 10 a, b Effect of storage period on cooking loss and cooking yield for SPI, NSPI, GLY and NSPI beef burgers at zero time and after 3 months.

GLY and NGLY burger samples. The NGLY burger sample gave higher cooking yield and lower cooking loss than the other burger samples at zero time and after storage period. These results may be due to the formation of ice crystals and changes in the burger pH that lead to protein denaturation and decrease in WHC according to Babjiet al [48]**.** During the freeze storage period there was an increase in cooking loss for all samples. These results agreed with Babji et al [48] who showed that soy protein can be used as functional ingredients in meat products, due to their ability to increase the water holding capacity and decrease the cooking loss. Freeze storage for a long period of time (3-6 months) is known to exert the negative effects on WHC and cooking loss in meat and meat products. Those findings were also supported by Smith et al [49] which indicated that utilization of soy protein in meat products can reduce the cooking loss when compared to 100% meat products. Tsao et al [50] indicated that soy proteins have a high binding ability with muscle proteins in restructured meat products; this would have high potential in the production of binders.

Sensory evaluation

　　As shown in Table 5 burgers processed from NSPI proteins and NGLY scored highly in all sensory attributes as compared with SPI, GLY and the negative control burgers at zero time. The highest score for juiciness belonged to NGLY (8.63) which was not significantly higher than NSPI and GLY burgers (8.5 and 8.25), respectively. The highest tenderness score was for GLY and NGLY burgers (8.1 and 8.3) these results may be due to the nano particles which increase water holding capacity and oil holding capacity due to their small size.

　　These results were in agreement with Singh et al [51] whose findings concluded that nanotechnology has the potential to offer benefits in the meat industry in the complete chain in the improvement of taste, odor and texture attributes of food products. In addition, the sensory attributes for all burger samples were decreased in the end of storage time (3 months).

　　Glycinin and nano glycinin burgers were of significantly ($P \leq 0.05$) more tenderness than the SPI and NSPI burgers at zero time and after 3 months (Tables 5 and 6). The negative control was the least tender and the driest of the beef burgers examined after the end of storage period. Overall acceptability was a significantly difference in SPI, NSPI, GLY and NGLY burgers compared with the negative control beef burger after 3 months. No significant difference observed in flavour for all beef burger sample after 3 months. These results agreed with findings of Taki [52] who found that the use of a functional blend gave a more juicier product. Carvalho et al [53] reported that the addition of soy proteins in beef burger formulas improved the texture, sensorial acceptance and cooking properties.

TABLE 5. Sensory evaluation for burger produced by adds SPI, NSPI, GlY and NGLY at zero time.

Beef sample	Color*	Juiciness*	Flavour*	Tenderness*	Appearance*	Overall acceptability*
Negative control	7.00 ± 0.50[b]	6.50 ± 0.50[b]	7.83 ± 0.28[a]	6.83 ± 0.21[b]	7.00 ± 0.50[c]	7.03 ± 0.05[e]
SPI	7.66 ± 0.28[ab]	7.166 ± 0.2[b]	8.00± 0.50[a]	7.26 ± 0.38[b]	7.32 ± 0.28[c]	7.43 ± 0.05[b]
NSPI	8.33 ± 0.76[a]	8.50 ± 0.50[a]	7.67± 0.28[a]	7.33 ± 0.15[b]	8.17 ± 0.28[ab]	8.00 ± 0.10[ab]
GlY	7.83 ± 0.28[ab]	8.25 ± 0.50[a]	7.17± 0.57[a]	8.17 ± 0.28[a]	7.50 ± 0.75[bc]	7.83 ± 0.15[b]
NGLY	7.83 ± 0.76[ab]	8.63 ± 0.25[a]	7.75± 0.31[a]	8.33 ± 0.76[a]	8 ± 0.9[a]	8.22 ± 0.35[a]

• 　　Means with different letters in the same column are significantly different ($P \leq 0.05$), ± standard error

TABLE 6. Sensory evaluation for burger produced by add SPI, NSPI, GlY and NGLY after 3 months.

Sample after 3 months	Color *	Juiciness*	Flavour*	Tenderness*	Appearance*	Overall acceptability*
Negative Control	6.25 ± 0.57[b]	6.50 ± 0.21[b]	7.83 ± 0.28[a]	6.83 ± 0.28[b]	7.00 ± 0.50[c]	6.87 ± 0.15[e]
SPI	7.25 ± 0.25[ab]	7.25 ± 0.25[b]	8.00 ± 0.50[a]	7.17 ± 0.28[b]	7.17 ± 0.18[c]	7.36 ± 0.04[b]
NSPI	8 ± 0.56[a]	8.75 ± 0.50[a]	7.67 ± 0.28[a]	7.33 ± 0.28[b]	8.17± 0.28[ab]	7.93 ± 0.10[ab]
GLY	7.25 ± 0.18[ab]	8.25 ± 0.08[a]	7.36 ± 0.57[a]	8.17 ± 0.28[a]	7.50 ± 0.53[bc]	7.765 ± 0.15[b]
NGLY	7.52 ± 0.76[ab]	8.50 ± 0.25[a]	7.83 ± 0.28[a]	8 ± 0.76[a]	8.32 ± 0.28[a]	7.984 ± 0.27[a]

*Means with different letters in the same column are significantly different ($P \leq 0.05$), ± standard error

Conclusion

High intensity ultrasound treatments (HUS) (400 W), changed the structure of soy proteins particles, decreased particle size of SPI particle and aggregation of GLY particle to nano scale (1-100nm). The functional properties (hydrophobicity, emulsion properties, solubility, water holding capacity and oil holding capacity) of SPI and GLY particles increased. Nano soy proteins and nano glycinin can serve as functional ingredients in decreasing cooking loss and shrinkage, increasing water holding capacity, and improving the sensory attributes of beef burgers. Application of nanotechnology in the food manufacturing process can improve the overall quality of the product.

Conflict of interest

The authors declare that they have no conflict of interest regarding the publication of this paper.

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بروتينات فول الصويا النانومترية لتحسين جودة برجر اللحم البقرى

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تعتبر جودة برجر اللحم التي يتم إنتاجه باستخدام اللحوم بنسبة ١٠٠٪ أقل جودة مقارنة بالبرجر المنتج باستخدام بروتينات فول الصويا. إن الاتجاهات الحديثة للتكنولوجيا النانو يقدم إضافات لتحسين خصائص الطهي للبرجر البقري. أجريت هذه الدراسة لتحسين خصائص الطبخ للبرجر المصنع من اللحم البقري من خلال استخدام جزيئات بروتين فول الصويا النانومترى وبروتين النانوجليسينين. تم استخدام الموجات فوق الصوتية عالية الكثافة لتحضير البروتينات النانومترية. بروتينات النانو التي تم الحصول عليها تم توصيفها باستخدام معرفة جهد ال زٔ ، توزيع حجم الجسيمات ، التبلور ، بالإضافة إلى الخصائص الوظيفية مثل القدرة على الاحتفاظ بالماء ، القدرة على الارتباط بالزيت ، خواص مقاومة الماء ، قابلية الذوبان وخواص الاستحلاب. تم تقييم جودة برجر لحوم البقر بمقارنة فقدان الطهي ، وعائد الطهي ، والانكماش ، ودرجة الحموضة مع المقارنة بالعينة الضابطة. أوضحت النتائج أن الإنكماش وفقدان الطهي قد انخفضا من خلال زيادة فترة التخزين في برجر اللحم البقري المحضرة من بروتينات النانو مقارنة مع البرجر المعد من بروتين الصويا والجليسينين النانومترى. أظهر بروتين نانو جليسينين أعلى إنتاجية الطهي بين جميع البروتينات وكان أعلى بكثير (0.05 ≥ P) من العينة الضابطة فى نهاية فترة التخزين ، كان الانكماش أقل بكثير (0.05 ≥ P) لبروتين الصويا النانومترى والنانو جليسينين (١٨ و ١٧,٨ ٪ على التوالي) من العينة الضابطة (٣٠ ٪). زادت اللدونة لبروتين الصويا النانومترى والنانو جليسينين (٤,٤٢ و ٤,٢٣ ، على التوالي) مقارنة مع عينات البرجر الضابطة (٣,٧) بعد ٣ أشهر. إضافة بروتينات الصويا النانومترية الى البرجر المصنع من اللحم البقري سوف يحسن من جودة الطهي لمنتجات اللحوم .