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# A study on protein extraction of noni dry and fresh fruit with polyacrylamide gel electrophoresis

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**Abstract**
The use of Polyacrylamide gel electrophoresis (PAGE) was apparently a universal biotechnological method used to separate organic macromolecules. These molecules usually include nucleic acids and proteins, all separated according to their size and electrophoretic mobility. In this study, the Sodium dodecyl sulfate Polyacrylamide gel electrophoresis (SDS-PAGE) and the Two-dimensional Polyacrylamide gel electrophoresis (2D-PAGE), were used for the first time to analyze protein present in both samples of noni fresh fruit (NFF) and noni dry fruit (NDF). Accompanied by the Trichloroacetic acid- acetone (TCA/Ace) adapted method, the separation of protein bands and spots was achieved successfully in this study. *Morinda citrifolia* Linn (noni) fruit, has been very useful for medicinal purposes and a good food source. For over 2000 years till today, more studies were focused in investigating the phytochemical composition of the fruit i.e., the protein composition of the noni pulp. Thus, we used the PAGE aided with the TCA/Ace method and isolated the protein bands of both NFF and NDF samples. The NFF showed 12 band-layers ranging from 10-130KDa and compared to NDF with only 5 band-layers with protein size range 15-50KDa. Moving on, the 2D-PAGE analysis of the NFF sample, a total of (18.55 ± 0.71) protein spots were seen with size ranging from 10-72 KDa.

**Keywords:** *Morinda citrifolia* Linn, TCA/Ace, SDS-PAGE, 2D-PAGE, protein

## Introduction
Herbal medicine has been utilized for centuries in every culture all over the world. *Morinda citrifolia* Linn or commercially known as noni, was one of the initial amongst Chinese herbs declared in ancient literature used for folk medicine. The noni fruit and leaves were always familiarly brewed and consumed as tea [1]. Recently, noni fruit became a good source of bioactive compounds unveiled by studies and so as a good source of food [2,3]. The noni plant has been seen farmed in tropical and coastal regions. These regions include, Latin America, Mexico, Columbia, Venezuela, Costa Rica, Panama, Kenya, Florida, West of India, Hainan China, Japan, Indonesia, Malaysia, Australia, Hawaii and, the Polynesian Islands [4]. The noni leaves, flowers, fruits, roots, and stems were exploited for food, medicine and sold in both local and large markets [5–7]. The fruit was consumed mostly as fermented juice, capsules as supplements and made into cheese for food. The fruit was also brewed for tea, edible fresh, with description of multiple, oblong, 5 to 7 cm long, with squelchy, tender and a cheesy fragrance [8–12]. Due to discovered nutraceutical and therapeutical properties of the noni fruit in 1996, the commercial noni juice was marketed as a dietary supplement and was later sanctioned in 2003, by the European Commission as a new food [13]. The Chinese government has also approved noni juice as a harmless novel resource and to be used as a functional food to be consumed to help improve human health [14]. Noni benefits to health were seen for; immunostimulatory, antitumor, antidiabetic [15,16], anti-obesity, anti-microbial and anti-septic, antiviral, leishmanicidal, anti-inflammatory, antinociceptive and pain-relieving, antioxidant, neuroprotective [17], abrasion healing [18], antiallergic, anti-dyslipidemia [19], antiangiogenic, antiemetic and anti-nauseant-gastric ulcer and esophagitis, antimutagenic, antipsychotic [20], antianxiety [21,22], photoprotective [23], anti-wrinkle and periodontal bone and soft-tissue rejuvenation [24–31]. Over the past decades, there were several prominent phytochemicals identified in noni that belong to structural compound groups which include; phenolic composites, anthraquinones, sugars, organic acids, alcohols, vitamins, flavonoids, iridoids, ketones, lignans, triterpenoids, nucleosides, sterols, fatty acids, and carotenoids. These compounds were identified using gas to liquid chromatography analysis methods [13]. Although, according to proteomics that governs PAGE in SDS-PAGE and 2D-PAGE, is still a remarkable field with applications highly developed to identify and study protein content in animals and plants which is of high demand at present, i.e., for noni fruit. The PAGE defines a procedure widely handled in biochemistry, forensics, genetics, molecular biology and biotechnology to separate biological macromolecules, usually proteins or nucleic acids, fitting suitable to their electrophoretic agility. As with all types of gel electrophoresis, molecules may be movable in their native state, preserving the molecules' higher-order make-up or a chemical denaturant may be induced to withdraw this structure and turning the molecule into a formless linear chain whose agility depends merely on its length and mass-to charge ratio. Nucleic acids like

urea were the most used denaturant agent [32]. For proteins, the SDS was an anionic detergent applied to protein sample to linearize proteins and to impart a negative charge to linearized proteins. A vital aspect to attain a good protein study is the institution of optimized extraction and protein process methodology. As importantly emphasized for the 2-DE analysis with complicated techniques and according to the sample preparation, protein display can be quite different specifying the need for well optimized procedures. These all includes repeatability and reproducibility [33,34]. Based on these requirements in these stages, investigation of different parameters, combinations using minimum amount of tissue with protocols for protein extraction from plant materials were evaluated with special attention to their capacity for removal of interferents [35]. According to past studies, the extraction protocols revealed large protein yield differences obtained with TCA/acetone (TCA/Ace) method with evidence to be the most efficient which allowed detection of protein bands and spots [35,36]. In this study we were able to show the TCA/ Ace method was applicable and able to separate and display protein bands from NFF and NDF for the very first time in SDS-PAGE and 2D-PAGE.

**Materials**

The noni dried fruit slices and fresh pulps were purchased from the Chinese medicine health and wellness research institute, Hainan, China. The noni plants were grown under tropical conditions (mean annual temperature: 20-35°C, minimum temperature: 12°C). The fresh fruits were washed and package in tight sealed zip locked bags and stored in -80℃ to be used in the experiments. For the dry product, pulps were washed, sliced and dried in sterilized heat pump dryer chambers for 1-2 days temperature 50-60°C and then packaged in tight sealed desiccator. (See Figure 1 A. Fresh noni fruit and Figure 1B. Dry noni fruit).

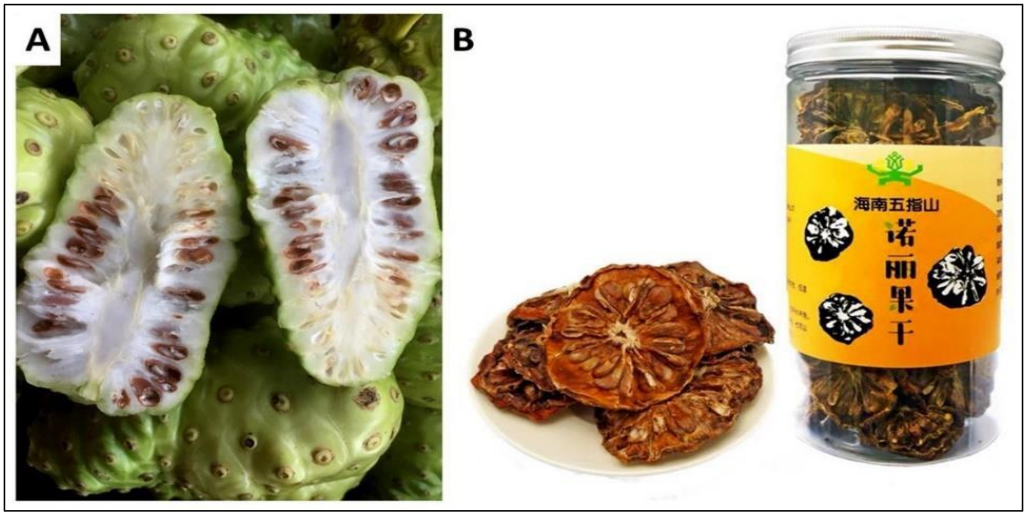


**Fig 1:** A. Fresh noni fruit B. Dry noni fruit

**Reagents**

Seen in Table 1 listed below are all chemical reagents needed for the pellet, protein retrieval and sample analysis used in this study. In the method section are all details of buffers needed to be prepped according to the individual volumes, concentrations and so as their individual storage conditions of each reagent respectively. In Table 2 are all instruments used for the extraction. Tables 3 listed are volumes of each component of the 16% SDS-PAGE for both the stacking and separation gels. Table 4 shows the IEF running sequence for the first dimension of the 2D-PAGE.

**Table 1:** Table of Reagents

| Reagent Name | Manufacturer |
|---|---|
| Acrylamide (Acr) | Alladdin, Shanghai (SH), China (CHN) |
| Beta Mercaptoethanol (β-ME) | |
| Bis-acrylamide (Bis-AC) | |
| 3-cholamidopropyl dimethyl-ammonio 1-propanesulfonate (CHAPS) | |
| Polyvinylpyrrolidone (PVP) | |
| Tris-base | |
| 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) | Alladdin, SH, CHN |
| Protease inhibitor (PI) | |
| Immobilized pH gradient (IPG) gel dry strip | Biorad, SH, CHN |
| Bromophenol blue (BPB) | Innochem Co., Ltd, SH, CHN |
| Commissive brilliant blue (G-250) | Macklin, SH, CHN |
| Commissive brilliant blue (R-250) | |
| EDTA disodium salt dyhydrate (EDTA-$Na_2$) | |

| | |
|---|---|
| Glycerol (Gly) | |
| Glycine (Glc) | |
| Magnesium Chloride (MgCl2) | |
| Mineral oil | |
| Paraffin oil | |
| Sodium 3-glycerophosphate (β- GP) | |
| Sodium Chloride (NaCl) | |
| Urea | |
| Deionized distilled water (dd-H2O) | Millipore Elix 3, SH, CHN |
| Distilled water (dH2O) | |
| Iodoacetamide (IAA) | Sangon biotech, SH, CHN |
| PG-Buffer | |
| 2-Propanol (2-POH) | Sigma, SH, CHN |
| Acetic- acid (Ac-A) | |
| Ammonium persulfate (APS) | |
| Butanol (BtOH) | |
| Ethanol (EtOH) | |
| Methanol (MeOH) | |
| Sodium dodecyl sulfate (SDS) | |
| TEMED | |
| Trichloroacetic acid (TCA) | |
| Phenylmethylsulfonyl (PMSF) | Solarbio, Beijing, China |
| Triton X-100 | |
| Thiourea | Titan Co., Ltd, SH, CHN |
| Protein Maker | Yajiang Biology Co., Ltd, SH, CHN |
| Sodium Vanadate (Na3VO4) | Yeasen biological Co., Ltd, SH, CHN |
| Agarose Molecular grade (Agar) | Yeasen, SH, CHN |
| Dithiothreitol (DTT) | Zhonghe Chemical Co., Ltd, CHN |

**Instruments**

**Table 2:** Table of instruments

| Instrument | Model number | Manufacturer origin |
|---|---|---|
| Food blender | MY-A1N | USA |
| Freezer (-4℃ to 20℃) | BCD-256KFB | SH, CHN |
| Water Milli-Q system | | Millipore Elix 3, CHN |
| Eppendorf centrifuge (50 mL tubes) | 5810R | CHN |
| Freeze dryer (Cool Safe) | Pro 55-9 | |
| Vortex (Gene 2) | G506E | USA |
| Image scanner III Epson | JI8I | Japan |
| Electronic scale (Mettler Toledo) | AL204 | CHN |
| pH calibrator | | |
| Eppendorf centrifuge (1.5/ 2 mL tubes) | 5415R | |
| Mini-pro vortex | ES-MCK6 | Yeasen, CHN |
| Dry thermostat | K30 | Aosheng Inst, CHN |
| Orbital shaker | TS-2 | Haimen Qilimber Inst, CHN |
| Vibra cells (Sonics) | VCX150 | USA |
| IPG Immobiline dry strip | 7 cm length, pH 3-10 NL | General healthcare, SH, CHN |
| PVDF Membrane | | |
| Biorad (Electrophoresis) | Power Pac HC | Singapore |
| Biorad (Protean IEF Cell) | | |

**Method**

***NFF and NDF pellet retrieval***

The NFF and NDF raw materials were diced and a net weight of 80g and 30g of each sample were first soaked in 100 mL of 2%PVPP buffer separately. [2%PVPP buffer:12 g PVP, 90 mL Tris-HCl (pH6.8),150 mL Gly, 3.6 g SDS, 3x β-ME (3 µL) topped with dd$H_2O$ and Stored in 4℃]. The sample was placed and settled in ice bath for 30 min. The samples were then blended, mortar grind and then rest to homogenize again for 30 min in ice bath. The supernatants were filtered out through the gauze fabric removing huge particles. TCA buffer (1:1 v/v) was then added to the filtered supernatants separately, vortex mixed then placed in -20℃ freezer for 24 hr. [TCA buffer: 600 mL of 10% TCA (w/v) in $H_2O$. Stored in 4℃]. The remaining insoluble matters (pellet) were partitioned by centrifuge at 3000 rpm at 4℃ for 30 min. (Note: supernatants were discarded gently to maintain the pellet integrity retained at the bottom of the vial). The pellets were then rinsed washed with 10 mL of wash buffer in ice-cool condition, homogenized vortex for 20 mins, then rest in the ice-bath for 30 min. [Wash buffer:

600 mL with 0.07% (v/v) of β-ME in Ace for wash solution. Stored in 4℃]. The samples were then centrifuged for 30 min in 4℃ at 3000 rpm. (Note: The wash cycle is repeated for 3-5 washes till supernatant discarded were colorless before proceeding to the next step). The samples were then placed in -80℃ for 24 hr, before placed in the freeze dryer vacuum desiccator at 4℃ for 48 hr for complete parching.

***NFF and NDF protein retrieval***

The NFF and NDF dry pellets of 30 mg from each was emerged in 500 µL of lysis buffer separately. Each sample were vortexed vigorously for 20 min and placed to rest in ice bath for complete homogenization for 30 min. [Protein lysis buffer: Components include 0.05% Triton X-100 (v/v), 2mM EDTA-$Na_2$, 25 mM HEPES, 400mM NaCl, 1.5 mM $MgCl_2$, pH=7.7 and a total volume 200 mL was prepped and stored in 4℃ freezer. (Note: 1 mL of lysis buffer was used with 1µL of 0.1M PMSF-dissolved with Isopropyl alcohol, 10 µL of PI, 10 µL of 1M DTT, 20 µL of 1M β-GP, 1 µL of 1mM $Na_3VO_4$ all dissolved with $H_2O$ separately and store in -20℃]. The samples were then subject to sonication [150 watt, 20 KHz, Amplitude 55% with Pulse 03/04] in ice-bath for 20 min. The protein-debris partitioning of samples was done by centrifuging at 7000 rpm in 4℃ for 1 min. Each sample supernatant was recovered to a new microtube and stored at -80℃ for further use for analysis. (Note: Sample must always be placed in cool to avoid protein denaturing).

***NFF and NDF 16% SDS-PAGE protein analysis***

Protein quantification: the total soluble protein concentration for both NFF and NDF samples was determined by using the Bradford method (1976) with the bovine serum albumin (BSA) done at room temperature and later checking for $OD_{595}$. [BSA is prepped 1 mg/mL dissolved in dd$H_2O$ and stored in 4℃]. The protein volume of 1 µL from each FNF and DNF was sampled into 100 µL of R-250 reagent in triplicates to measure the protein concentration at $OD_{595}$. After calculating the protein concentration using the standard curve, the protein sample of 20 µg, 50 µg and 70 µg from each NDF and NFF protein samples was added into a new tube. A 1/3 of x4 loading buffer was later added together, vortexed, centrifuged then heated at 95℃ for 3 min then was loaded into the gel chambers ready to run sequence.

SDS-PAGE gel prep: the assembling of all the gel mold components was done as instructed by the Bio-Rad manual. The 16% separating gel mixed was filled into the glass frame and topped with dd$H_2O$ and left to solidify for 30 mins in room temperature (See Table 3). The topped dd$H_2O$ was then discarded and the mixed stacking gel was poured in, followed by the placement of the chamber frame tightly sealed on top and settle for 30 min to solidify at room temperature. (Note: Avoid bubbles trapped in the sample chamber space).

The gel glass was assembled into the cartridge and placed into the buffer dish. TGSx1 electrophoresis running buffer was poured halfway to submerge the gel covering the chamber frame. [(TGS) 10x electrophoresis buffer: 144.2 g of Glc, Tris-HCl 30.2 g, SDS 10 g were dissolved in 1 L of dd-$H_2O$, magnetically stirred, stored at room temperature]. After the sample were loaded into the chamber frames, the lid was placed with electrodes assembled to the proper channels then proceeded with the running sequence. Gel run sequence; Stacking gel run sequence 1: 30 min at 60 volt and separating gel sequence 2: 1.5 hr at 120-volt, 3 Amp, 300 Watt. After the end of sample running sequence, the gel was removed from the frame then washed with dd$H_2O$. Gel sample was stained with R-250 stainer for 30 min under low agitation. [Gel R-250 stainer: 1g of R-250, 100 mL of EtOH, 250 mL of 2-POH with d$H_2O$ was prepped to a total volume of 600 mL, stored at room temperature]. The gel was then washed with dd$H_2O$ then with the destain solution for 24hr at low agitation. [Gel destain solution: 100 mL of Ac-A, 50mL of EtOH and d$H_2O$ topped with 850 mL total volume]. The gel sample was then digitalized in colored and analyzed with Gray scale for target protein bands observation. All experiments were done in n-independent tests, and the experimental data are displayed in the form of mean and standard deviation (mean ± SD). Data were analyzed with t-student value, and one-way ANOVA ($P < 0.05$) indicating a significant difference.

**Table 3:** Preparation of separation gel and stacking gel

| Reagent (Volume) | Stacking gel | Separating gel 16% |
|---|---|---|
| 30% Acr-Bis (mL) | 0.33 | 4.4 |
| 1.5 M Tris-HCl pH 8.8 (mL) | - | 2.2 |
| 0.5 M Tris-HCl pH 6.8 (mL) | 0.5 | - |
| 10% SDS (µL) | 20 | 80 |
| 10% APS (µL) | 15 | 80 |
| TEMED (µL) | 2.5 | 8 |
| H2O (mL) | 1.15 | 1.4 |

***NFF 2D-PAGE isoelectric focusing analysis***

The NFF protein was extracted and quantified as mentioned above in the previous section. (See the SDS-PAGE NFF and NDF protein retrieval and protein analysis section). A sample of 200 µg – 500 µg from the FNF protein was added to SB, substituting for the lysis buffer making a total of 125-150 µL of sample in a new vial. [Solubilization buffer (SB): 7M Urea, 2M Thiourea, 4% CHAPS, 2% IPG buffer and 0.3% DTT, was dissolved in d$H_2O$. (Note: Urea, Thiourea, CHAPS, was prepped and store in -20℃m separately. A 0.0001% Bromophenol blue (BPB) (w/v) was prepped and stored in 4℃ freezer)]. The sample was then vortexed for 20 mins and semi centrifuged and replaced in the ice bath. A 1.5µL of BPB was added into the sample then and vortexed mixed before stored into the ice bath for 20-30 min. The sample was then cooked for 2 min at 95℃, vortexed and semi-centrifuged before pipetted into the sample tray chamber. The gel strip was then placed on top of sample, gel

face down on top of the sample for the hydration process. Upon gel strip placement, the strip was gently laid on top of the aliquot avoiding the trapping of bubbles. (Note: the IPG strip was placed out at room temperature for 20-30 min before proceeding to the hydration process with the sample). The gel was placed out to rest for gel strip hydration with the sample for 1 hr at room temperature then covered with 1-2 mL of paraffin oil. The sample tray was then placed into the chamber with electrodes aligned and proceed on with the sample running sequence.

First dimension separation run sequence: the IEF sequence of sample for gel complete rehydration was done overnight with IPG-strip/NFF sample at 20℃ using the Biorad Protein IEF Cell. The gel sequence was inputted (See Table 4) and was left to run for 24-25 hr. After the running sequence was completed, the sample was gently removed, and the gel-less side was gently wiped down clearing any left-over paraffin oil residuals. The gel-less side was washed with $ddH_2O$ and again gently dried with filter paper. The sample strip was then transferred onto a new tray slot with the gel-side facing upwards. The sample completely submerged with 4 mL of RB solution with the gel side-up at low agitation. [Reduction buffer (RB): 0.05M Tris-HCl (pH 8.8), 6M Urea, 30% Gly, 2% SDS all stored in -20℃ and 1% DTT (Note: DTT was added prior to use in the time of the experimental test)]. The gel-side was then gently dried with the filter paper then transferred into new tray slot with 4 mL of AB for alkylation [Alkylated buffer (AB): 0.05M Tris-HCl (pH 8.8), 6M Urea, 30% Gly, 2% SDS, 2.5% IAA, 0.002% BPB all stored in -20℃ (Note: IAA and BPB were added prior to use in the time of the experiment test)]. Both processes were done at room temp for 15 min with gentle agitation.

Second dimension: the separation was done with 16% SDS PAGE separating gel (See sections above on the 16% SDS-PAGE preparation). First the IPG-NFF sample strip equilibrated were horizontal placed on top of the separating gel aligning with the positive and negative nodes of cartridge (from left to right direction). Sample gel and the separating gel was sealed with a concentrated gel sample of 0.5% agar (w/v) with 3 µL of 0.0001% BPB, making a total of 4mL. (Note: press down to avoid bubbles trapped in between both IP sample gel with the electrode poles aligned). After gel place at room temperature to solidify for 30 mins. The sample was then subject to sequence run similarly to the above sequence to the SDS-PAGE sample. The sample was later stained for 30 mins with the R-250 stainer with slow agitation. The retaining of the gel sample then done over night under low agitation. The gel sample was digitalized and later analyzed. All gel samples can be stored at 4℃ for future reference. All experiments are done in n-independent tests with mean and standard deviation (mean ± SD). Data were tested by student t-value and analyzed by one-way ANOVA, ($P < 0.05$) indicating a significant difference.

**Table 4:** IEF Hydration of Sample Run Sequence

| Sequence | Voltage (V) | Mode | Time (hr) | Function |
|---|---|---|---|---|
| S0 | 50 | Linear | 13/ 20℃ | Rehydration |
| S1 | 250 | Linear | 0.5 | Desalting |
| S2 | 500 | Rapid | 0.5 | Desalting |
| S3 | 4000 | Linear | 3 | Boost voltage |
| S4 | 4000 | Rapid | 20000 - V/hr | Isoelectric focusing |
| S5 | 500 | Rapid | 8 hr | Protection procedure |

**Results**

Seen in table 5, were the NFF and NDF pellet retrieval from the fruit raw material used.

**Table 5:** Pellet Retrieval and Protein Recovery

| Sample | Fruit weight (g) | Pellet collected (g) |
|---|---|---|
| NFF | 84.87 ± 9.58 | 0.45 ± 0.12 |
| NDF | 36.04 ± 1.13 | 0.48 ± 0.02 |

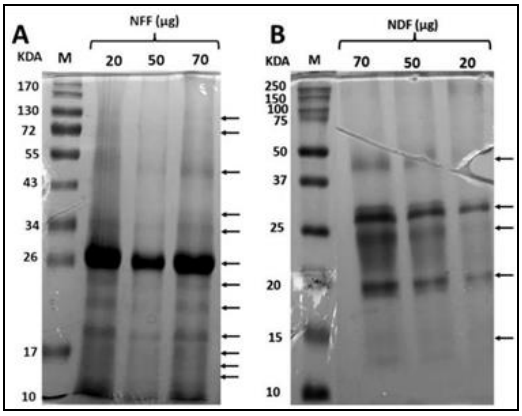


**Fig 2: (A)** NFF protein bands from 10-130 KDa, (**B**) NFD protein bands from 15-50KDa in16% SDS-PAGE with loading protein weight 20 µg, 50 µg and 70 µg. Samples n=5

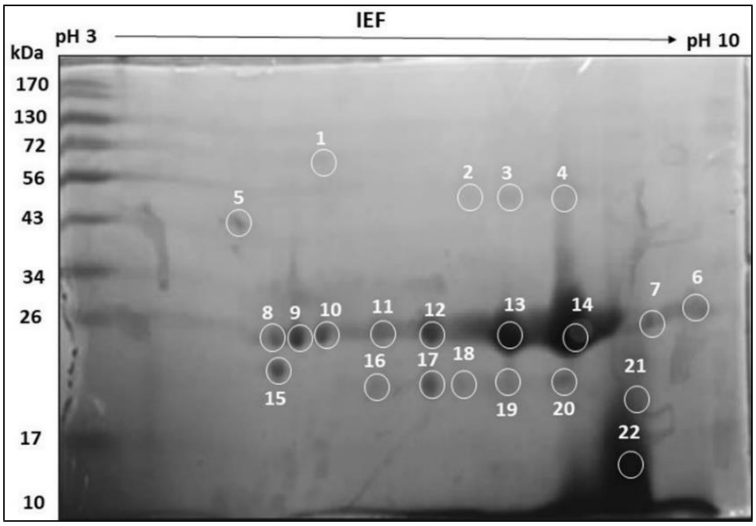


**Fig 3:** 16% 2D-PAGE of NFF protein sample. The 2D-PAGE tacked NFF protein spots resolved within an IE pH range of 10-72 KDa. Samples n=3

**Discussion**

The NFF and NDF pellet retrieval with the adapted TCA/Ace method has performed very tremendously efficient in obstructing major obstacles as viewed in the reproducibility of the SDS-PAGE analysis. The overview of the 2D electrophoresis was successfully achieved especially for the NFF protein spot analysis. The study confirms that most critical steps were the protein extraction and sample preparation, especially in plants. Lack of proper extraction procedure may stage low protein content and produce some sorts of subordinate metabolites [37]. Past protein extraction studies impose a lot of views which were largely employed with emerging techniques, integrating different analytical methods to identify very essentials steps. These steps were studied extensively to obtain good results [38,39]. It is known that the acetone-based methods efficiently concentrate proteins while removing salts, sugars and some lipids [40]. It was proven as relatively faster in this study in collaboration to others who had mentioned previously [35]. In this work with the extensive research and integrated adapted methods to the TAC/Ace, we used the least amount of NDF and NFF plant material required (0.8 g NFF and 0.3 g for NDF) in all extractions to obtain the above results. The result showed that we had managed to use the TCA/Ace method and implementing a 2% PVPP to both NFF and NDF in the protein preliminary stages and was seen to be successful, compared to past studies using a 10% PVPP which was also used prior in this study but was found not successful [35,41]. The analysis pellet yield showed that the TCA/Ace method was the most efficient for NFF (0.48 per 36.04 mg) and NDF (0.45 per 84.87 mg) extrapolating a 1.3% and 0.05% pellet recovery rate respectively. According to our result seen in Figure 2, it shows the 16% SDS-PAGE of both NFF and NDF, several levels of protein bands were able to be separated with protein size range from 10-130 kDa respectively. These bands were seen much clearly with large range of size compared to past electrophoretic profile of noni extract. These protein bands ranged from 14 to 55 kDa and was analyzed from cheese manufactured from noni puree extract [42]. Further analysis to the 16% SDS-PAGE for NFF, there were 12 protein bands with protein size range of 10-130 KDa. Compared to the NDF it was seen to result with 5 protein bands with size ranges from 15-50 KDa. On basis of most visible protein bands and protein size range, the NFF with more band appearance, the NFF was subjected for 2D-PAGE test. The 2D-PAGE profile of the NFF obtained from the TCA/Ace method resolved with spots plotted within an IEF pH range of 3-10 in protein level sizes from 17-72 KDa. The minimal spots count of (18.55 ± 0.71) was compared among 3 sample tests separately (Figure 3). To conclude we had managed to develop a standardized 2D-PAGE to obtain accurate protein maps for NFF. Using 13 mg of pellet with 500 µL of our adapted lysis buffer to plant tissue, we were able to employ 200-300 µg loading protein sample for each reproducible gel test in the study. Amongst the precautioned of this noni fruit study analysis, was how important alteration in the TCA/Ace method was to preserve the extraction in cold during all stages, preventing hydrophobic interfaces and complexes formation, thus augmenting the sample solubilization which was a very important factor to consider. According to these results obtained under the optimized and adapted tested procedure, a conclusion revealed that by using TCA/Ace extraction resulted in the best mining efficiency and greater protein separation using both SDS-PAGE and 2D-PAGE in both dry and fresh noni fruit. Therefore, the use of TCA/Ace extraction protocol combined with the IEF protocol described here for extraction and separation may facilitate noni fruit and plant parts in proteomic research with guided protocols to further deeper plant research studies and other plant species.

**Limitations**

The analysis of individual protein band and spot by mass spectrometry will be conducted to confirm each protein or nucleic acid. The use of SDS-PAGE and 2D-PAGE electrophoresis study should do with fresh fruit seed, skin and flesh separately and further studies should implement the used of additional methods in different elements from noni fresh fruit.

**Conflicts of interest**
The authors declare no conflict of interest.

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