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| Please use this form to clearly and concisely report on project progress. The information included should reflect quantifiable results that can be used to evaluate and measure project success. Comments should be limited to the designated boxes. Technical reports, no longer than 4 pages, may be attached to this summary report. | |
| Project Number: | AWD-100936 |
| Project Title: | Novelnew functional edible protein films from soybean using innovative 3D printing technology. |
| Organization: | Department of Food Science, University of Arkansas. |
| Principal Investigator Name: | Dr. Navam Hettiarachchy |
| Other investigators: | Drs. Leandro Angel Mozzoni, Pengyin Chen. |
| Report Period: | June 16, 2021 to September 15, 2021. |

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| Project Status: On-going (Year 1 of 2)(What key activities were undertaken and what were the key accomplishments during this quarter? Please use this field to clearly and concisely report on project progress). **Objectives for the project:**  1. Prepare soy protein isolate and hydrolysates from soybean seeds grown in Mid-South (lines AR-R11-7999 from Arkansas, MO-S17-19874R and MO-S17-17168 from Missouri).  2. Optimize conditions and prepare homogeneous protein film solutions with soy protein isolate, and investigate flow properties to prepare edible film.  3. Optimize conditions and extrude soy protein film using 3D printing technology, investigate the physical properties of the extruded films for color, tensile strength, and antioxidant activity of the extruded film.  **Accomplishments this quarter**:  **Objective 1:**  A total of three lots of soybean grown in mid-south (lines AR-R11-7999 from Arkansas; MO-S17-19874R and MO-S17-17168 from Missouri) (Figure 1) were provided by Drs. Mozzoni and Chen respectively.  **Figure 1**: Soybean lots used for isolation of protein.    **Preparation of defatted soybean meal:**  Soybean seeds were ground to a coarse powder using a pulverizer (Fritsch GmbH, Germany). The resulting coarse product was ground to a fine flour using a vitamix grinder, passed through a 60-mesh screen to obtain uniform particle size, and suspended in *n*-hexane (1:2, w/v), stirred for 3h at ambient conditions to remove the oil. The resulting suspension was filtered under vacuum, and defatted again to remove last traces of oil, filtered under vacuum and the residue was dried overnight, at ambient temperature, under a hood, ground and stored at 5 °C.  **Preparation of soy protein isolate:** Defatted soy meal (60 mesh) in a beaker was suspended in DI water (1:9 w/v). The pH of the solution was adjusted to 9.5 using 3N NaOH solution. The resulting suspension was stirred for 3h to release the proteins in the soy meal, pH was checked every 30 minutes, adjustments were made as needed. The resulting suspension was centrifuged at 3000xg for 15 minutes to remove the carbohydrates, fibers and leftover unextracted proteins. This Insoluble residue was re-extracted to solubilize the remaining proteins. The soluble supernatant containing the proteins was adjusted to 4.5 (isoelectric pH) to precipitate the protein. The precipitated proteins were centrifuged at 7000xg for 75 minutes after storing at refrigerated temperatures overnight to facilitate further precipitation of protein. The residue containing the protein was washed with water at pH 7.0 and freeze dried to obtain the protein isolate. Soy protein isolates 1 and 2 were combined to obtain the final protein isolate (Figure 2).  **Figure 2**: Optimized method for isolation of protein from soybean seeds using alkaline extraction.    **Protein content determination:** Protein contents were determined by Kjeldahl method. The protein contents on the dry weight basis of AR-R11-7999, MO-S17-17168, and MO-S17-19874R soybean seeds were 40.0, 39.1, 39.9, and in the protein isolate 84.5, 84.7, 87.3 %, respectively (Table 1). The Missouri soybean lot MO-S17-19874R demonstrated higher protein content both in the seeds and the protein isolates compared to the other two soybean lots (Table 1).  The protein contents in the soybean and the isolated soy protein and the moisture content were determined using the method reported by Rayaprolu et.al. (Rayaprolu *et.al.* 2015).  Rayaprolu, S; **Hettiarachchy, N**; Horax, R; Satchithanandam, E; Chen, P; and Mauromoustakos, A, (2015), Amino acid profiles of 44 soybean lines and ACE-I inhibitory activities of peptide fractions from selected lines. *JAOCS*, 92: 1023-1033.   |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | | Lot number | Protein content (% g/100g) by Kjeldahl method (dry weight basis) a,b | | | | | | | Ground soybean flour | | | Protein isolate | | | | Protein content (as is) | Moisture content | Protein content (dry weight basis) | Protein content (as is) | Moisture content | Protein content (dry weight basis) | | AR-R11-7999 | 37.2 ± 0.4 | 7.1 ± 0.1 | 40.0 ± 0.4 | 82.3 ± 2.4 | 2.6 ± 0.1 | 84.5±2.5 | | MO-S17-17168 | 36.5 ± 0.9 | 6.3 ± 0.3 | 39.1 ± 1.0 | 82.7 ± 2.5 | 2.4 ± 0.5 | 84.7±2.9 | | MO-S17-19874R | 37.5 ± 0.5 | 5.9 ± 0.1 | 39.9 ± 0.5 | 84.9 ± 0.5 | 2.7 ± 0.2 | 87.3±0.5 |   **Table 1**: Protein contents of ground soybean flour and protein isolate.  a Data are represented as mean ± standard deviation from three independent experiments.  b Protein content was determined from the total nitrogen determination by Kjeldahl method using Kjeldahl factor 6.25.  **Objective 2:**  Research is in progress preparing protein hydrolysates using food grade *alcalase* enzyme and other novel treatments to prepare protein films for 3D printing.  **Non-technical summary:**  Arkansas-grown and Missouri-grown soybean seeds were provided by Drs. Mozzoni (AR) and Chen (MO). The soybeans were ground and passed through a 60 mesh, defatted, and protein isolate was prepared. The protein contents on the dry weight basis of AR-R11-7999, MO-S17-17168, and MO-S17-19874R soybean seeds were 40.0, 39.1, 39.9; and in the protein isolate 84.5, 84.7, 87.3 %, respectively.  The Missouri soybean lot MO-S17-19874R gave slightly higher protein content both in the seeds and the protein isolates compared to the other two soybean lots. Treatments and conditions are being optimized to prepare functional proteins and hydrolysates from the isolates. These will be evaluated for preparing film, prepared using the emerging 3D printing technology. |