Scientific Bulletin. Series F. Biotechnologies, Vol. XXIV, No. 1, 2020
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

EFFECT OF VARIOUS EXTRACTION METHODS ON PEANUT PROTEIN EXTRACTION EFFICIENCY

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Abstract

Peanut allergy is one of the most common food allergies. Peanuts can cause a severe, potentially life-threatening allergic reaction (anaphylaxis). In order to improve consumer protection, our study is geared to quantification of proteins through several extraction methods. Fried peanuts samples were firstly defatted by stirring with pre-cooled acetone and 0.07% β-mercaptoethanol and then extracted with different buffers. The effect of various extraction procedures on the extraction efficiency for peanut protein was investigated by Lowry assay. Bovine serum albumin (BSA) was used as standard. Our work shows that the results depend on extraction method used.

Key words*: Lowry assay, peanut allergy, peanut extraction.*

INTRODUCTION

Food-induced allergy represents a public health problem spread worldwide affecting adults and children with a rising growth. According to Food and Agriculture Organization, the most common allergenic foods include peanuts, milk, soy, eggs, nuts, fish, shellfish, and wheat. They are responsible for 90% of the total numbers of food allergies (Ekezie, Cheng and Sun, 2018; Pele and Campeanu, 2016).

Among allergic foods, peanuts (*Arachis hypogaea*, fam. Fabaceae) represent the main causative factor of the most severe allergic reactions, including allergic and anaphylactic shocks (Xiaowen et al., 2019). Peanuts are used worldwide in food industry for oil production, peanut butter, cake decoration, roasted peanuts and snack products, extenders in meat product formulations, soups and desserts (Zhao et al., 2012). A major concern of health organizations is that even trace amounts of peanut can induce serious allergic reactions for certain people, for example threshold doses are as low as 100 μg of peanut protein (Al-Muhsen et al., 2003). Also, often peanut allergies are persistent throughout the lifetime, and only 20% sensitive individuals can overgrow it (Skolnick, 2001). Currently, some therapies have been introduced to reduce the prevalence of peanut allergy including strict avoidance and rescue medication upon accidental exposure to peanuts, oral immunotherapy, modifying or removing allergens from foods, etc. (Sitton and Temples, 2018; Bavaro et al., 2018).

Peanut kernels contain lipids, proteins and fibers along with some amount of carbohydrate, vitamins, and minerals (Pi et al., 2019). Overall, seventeen protein allergens have been identified in peanuts and are listed by the Allergen Nomenclature Sub-Committee of the International Union of Immunological Societies (Subcommittee, 2020), named Ara h1 to Ara h17. Among these allergens, Ara h1, Ara h2, Ara h3 and Ara h6 are more abundant and associated with severe allergic reactions (Zhuang and Dreskin, 2013).

Proteins need to be extracted efficiently to ensure that an accurate representation of allergenic proteins from the source material is obtained. Therefore, the selection of an adequate method of extraction represents a major step for

further investigations. In order to obtain an optimized peanut extraction, several extraction conditions must be tested and their effects on protein characteristics need to be investigated (Ma et al., 2010). The factors that affect the extractability of proteins include: the extraction buffer (nature, pH, ionic strength), the solid-to-liquid ratio, the extraction temperature, the extraction duration, etc. (Walczyk et al., 2017; Kain et al., 2009, Kim et al., 2011; Poms et al., 2004). Usually, the main goal in optimization of protein extraction conditions is to obtain as much protein as possible. Several methods have been employed to assess the total protein content in samples like: UV detection, Lowry method, BCA assay, Bradford method, biuret method, etc. (Nishi et al., 1985; Zheng et al., 2017). The Lowry method is one of the most used procedures in order to quantify proteins, due to its sensitivity, good reproducibility, easy to apply (Upreti et al., 1988; Pires-Oliveira and Joekes, 2014).

Herein we investigated the influence of several parameters on the extractability of protein, such as: extraction buffer (pH and molarity), solid-to-liquid ratio, temperature of extraction, and duration of extraction. The protein extractability, assessed as total protein content, was quantified by Lowry assay using bovine serum albumin (BSA) as standard protein.

This knowledge will be valuable for the optimization of peanut protein extraction for medical researchers and peanut breeders.

MATERIALS AND METHODS

Materials: β-mercaptoethanol, Tris-HCl, Folin Ciocalteu reagent, sodium carbonate, sodium hydroxide, copper sulfate and trisodium citrate were purchased from Sigma-Aldrich, Germany. BSA (synthesis grade, ≥ 95%) was obtained from Merck, Germany. Acetone used was analytical grade.

Peanut sample preparation

Fried peanuts were purchased from a local supermarket (Figure 1), washed with distilled water and drained at room temperature.

Figure 1. Peanut Sample (local market)

Protein extraction and characterization

Protein extraction

Defatted peanut powder was prepared using the procedure described by Zhou et al. (Wu et al., 2016). Fried peanuts were milled with a grinder to obtain a milled powder. The peanut powder was defatted by stirring with pre-cooled acetone and 0.07% β-mercaptoethanol (1:5 w/v ratio) for 2 h at 4°C. Pellets were then filtered by a vacuum filter. The defatting process was repeating three times. The defatted powder was dried in an oven at 40°C and stored at - 20°C. The obtained defatted powder was further used at extraction of peanut protein. Protein was extracted using five different methods (Methods 1-5) varying several parameters like: extraction buffer, solid-to-liquid ratio, temperature of extraction and duration of extracti
on.

Method 1: Peanut protein extract was obtained by mixing the defatted peanut powder with 20 mM Tris-HCl, pH 7.2 with ratio of 1:10 (w/v) at 25°C for 2-6 h. The crude extract was cleared by centrifugation at 10 000 rpm for 30 min at 4°C and the supernatant was collected.

Method 2: Peanut protein extract was obtained by mixing the defatted peanut powder with 20 mM Tris-HCl, pH 7.2 with ratio of 1:10 (w/v), and stirred at 4°C for 2-6 h. The crude extract was cleared by centrifugation at 10 000 rpm for 30 min at 4°C and the supernatant was collected.

Method 3: Peanut protein extract was obtained by mixing the defatted peanut powder with 20 mM Tris-HCl, pH 8.2 with ratio of 1:10 (w/v), and stirred at 4°C for 2-6 h. The crude extract was cleared by centrifugation at 10 000 rpm for 30 min at 4°C and the supernatant was collected.
Method 4: Peanut protein extract was obtained by mixing the defatted peanut powder with 50 mM Tris-HCl, pH 7.2 with ratio of 1:10 (w/v), and stirred at 4°C for 2-6 h. The crude extract was cleared by centrifugation at 10 000 rpm for 30 min at 4°C and the supernatant was collected.
Method 5: Peanut protein extract was obtained by mixing the defatted peanut powder with 20 mM Tris-HCl, pH 8.2 with ratio of 1:20 (w/v), and stirred at 4°C for 2-6 h. The crude extract was cleared by centrifugation at 10 000 rpm for 30 min at 4°C and the supernatant was collected.

Protein quantification

Extraction efficiency for peanut protein was investigated by Lowry assay. BSA was used as standard. In short, for the Lowry assay 0.5 ml of sample extract were incubated with 0.5 ml Lowry reagent at 25°C for 10 minutes; then were added 1.5 ml Folin Ciocalteu reagent (1:10 v/v) and kept for 30 minutes at room temperature. The absorbance was measured at 760 nm as compared to blank using an UV-Vis spectrophotometer (Jasco V630, Germany).
The Lowry reagent used was prepared mixing solution A with solution B 10:1 (v/v). Solution A: 10% sodium carbonate in 0.5% sodium hydroxide. Solution B: 0.5% copper sulfate in 1% trisodium citrate.

Statistical Analysis

Extraction experiments and protein content determination assessed by Lowry method were carried out in duplicate and, respectively triplicate. All data are expressed as the mean ± standard deviation.

RESULTS AND DISCUSSIONS

As peanut is a popular food and an important source of proteins and food oil worldwide, extraction represents an important step in the acquisition of target compounds from various materials (Kain et al., 2009; Sharma et al., 2002). Hence, the knowledge of protein extractability and quality is a key factor in selecting particular proteins for possible medical research and food applications (Poms et al., 2004; Jiang et al., 2010).
A challenge to overcome when studying proteins is the selection of the most appropriate method of protein extraction. Many factors affect the extractability of proteins.
For the selection of optimal extraction conditions of peanuts the influence of several parameters on the protein extractability (extraction buffer - pH and molarity, solid-to-liquid ratio, temperature of extraction and duration of extraction) need to be investigated. The protein extractability was assessed by Lowry assay.
The Lowry method was chosen for the protein quantification due to its sensitivity and good reproducibility. Also it is a very easy method to apply in laboratory. The peanut extraction conditions are listed in Table 1.

Table 1. Peanut extraction conditions

Sample name	Extraction conditions
I.1	Tris 20 mM; pH 7.2; 1:10 (w/v); 25^0C; 2 h
I.2	Tris 20 mM; pH 7.2; 1:10 (w/v); 25^0C; 4 h
I.3	Tris 20 mM; pH 7.2; 1:10 (w/v); 25^0C; 6 h
II.1	Tris 20 mM; pH 7.2; 1:10 (w/v); 4^0C; 2 h
II.2	Tris 20 mM; pH 7.2; 1:10 (w/v); 4^0C; 4 h
II.3	Tris 20 mM; pH 7.2; 1:10 (w/v); 4^0C; 6 h
III.1	Tris 20 mM; pH 8.2; 1:10 (w/v); 4^0C; 2 h
III.2	Tris 20 mM; pH 8.2; 1:10 (w/v); 4^0C; 4 h
III.3	Tris 20 mM; pH 8.2; 1:10 (w/v); 4^0C; 6 h
IV.1	Tris 50 mM; pH 7.2; 1:10 (w/v); 4^0C; 2 h
IV.2	Tris 50 mM; pH 7.2; 1:10 (w/v); 4^0C; 4 h
IV.3	Tris 50 mM; pH 7.2; 1:10 (w/v); 4^0C; 6 h
V.1	Tris 20 mM; pH 8.2; 1:20 (w/v); 4^0C; 2 h
V.2	Tris 20 mM; pH 8.2; 1:20 (w/v); 4^0C; 4 h
V.3	Tris 20 mM; pH 8.2; 1:20 (w/v); 4^0C; 6 h

Effect of buffer on protein content (pH and molarity)

The effect of buffer on protein content is presented in Figure 2 and Figure 3.

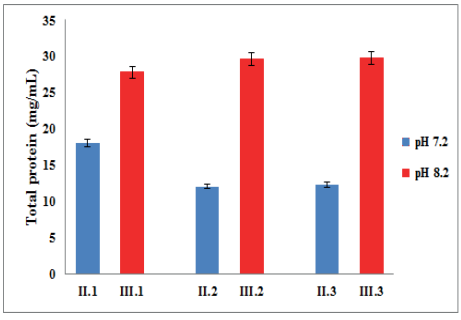

Figure 2. Effect of pH on total protein extraction

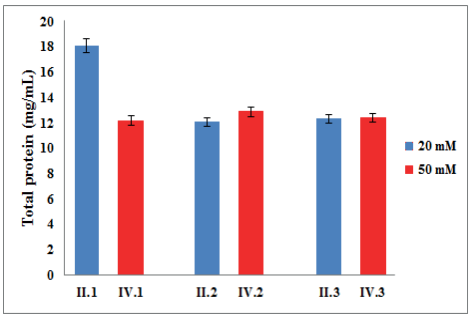

Figure 3. Effect of molarity on total protein extraction

It was observed the pH had a strong impact on the protein content in all samples. By increasing the pH value of the buffer from 7.2 to 8.2 the protein extractability increased as well. Our results were in agreement with the work of Shridhar et al. (2009) who reported that the protein extractability from edible nuts was about two times higher at pH 8.45 than at pH 7.2.

The molarity of buffer influenced the protein content in a lesser extent than the buffer pH. An exception was noticed at sample II.1 (Tris 20 mM; pH 7.2; 4^0C; 2 h) witch presented significantly higher value than sample IV.1 (Tris 50 mM; pH 7.2; 4^0C; 2 h).

Effect of solid-to-liquid ratio on total protein extraction

The effect of solid-to-liquid ratio of extraction on protein content is shown in Figure 4.

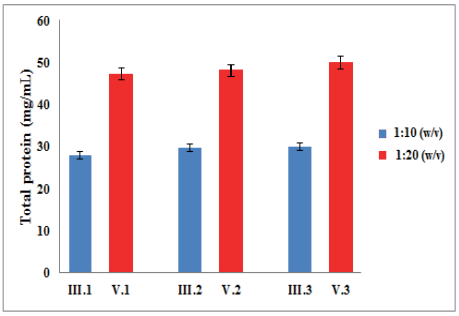

Figure 4. Effect of solid-to-liquid ratio on total protein extraction

It was observed that increasing the solid-to-liquid ratio from 1:10 (w/v) to 1:20 (w/v) the protein content increased in all samples.

These results were expected, because a higher solvent content leads to lower viscosity of the solution, thereby promoting molecular diffusion and facilitating protein extraction.

Effect of temperature of extraction on total proteins

The effect of temperature of extraction on protein content is shown in Figure 5.

In the case of the first two samples, the best results had been shown for sample II.1 (Tris 20 mM; pH 7.2; 4^0C; 2 h).

For the other samples significant differences were not observed.

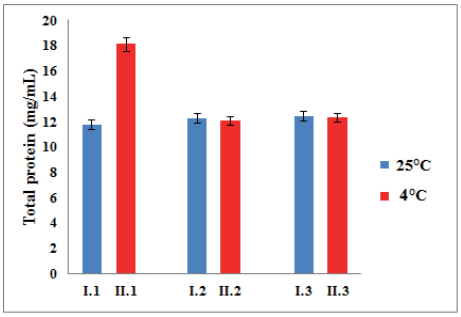

Figure 5. Effect of temperature on total protein extraction

Effect of duration of extraction on total proteins

The effect of duration of extraction of extraction on protein content is presented in Figure 6.

In the case of method I, III, IV, V slightly better results were obtained at a higher extraction time, while for method II the best extraction time was at 2 h.

Several papers reported that usually shorter extraction duration is preferred to minimize protein degradation (Kain et al., 2009).

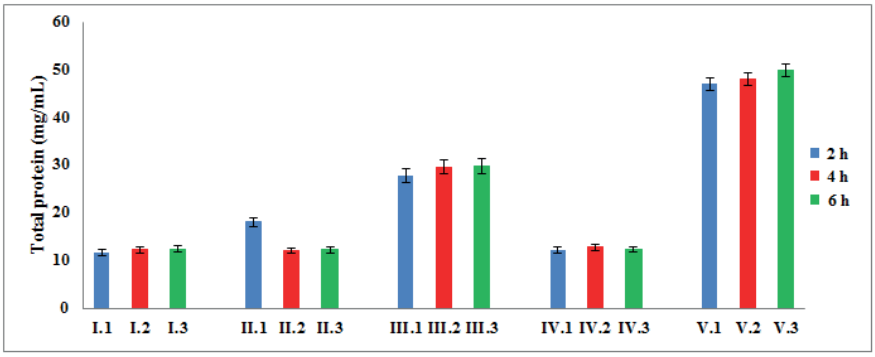

Figure 6. Effect of duration of extraction on total protein extraction

CONCLUSIONS

In this study, the influence of several extraction factors was assessed in order to identify the conditions that resulted in improved protein extractability.

Protein content increased significantly with increased pH and solid-to-liquid ratio of extraction. Buffer molarity, temperature of extraction and duration of extraction influenced the protein content in a minor extent.

These results present valuable information for the optimization of peanut protein extraction for medical researchers and peanut breeders.

Further investigations are needed to quantify the allergenic proteins from peanuts.

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