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EXTRACTION AND CHARACTERIZATION OF PROTEIN FROM *Vignamungo* AND ITS APPLICATIONS IN FOOD

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ABSTRACT

Pulses are annual legume crops, harvested for their seeds. Proteins are essential component of diet performing multifarious role in human body. Present project was an attempt to extract and characterize legume protein for their functional properties. Black gram with and without seed coat were analysed for their protein, moisture, ash and fat. Protein contents were 4.12 and 4.10 respectively. Black gram with seed coat exhibited highest amount of protein, fat, moisture and ash. High foamability of 67.8% and stability of 100% were obtained in water andsaline extracts when maintained at 25˚C. High foamability of 55.4% was obtained in the water extract when it was full fatted and heat treated. High foam stability of 100% was obtained in the saline extract when it was de-fatted and heat treated. Foaming capacity of flour suspensions at pH 7, 9 and 11 varied significantly from 24.33 to 33.97%. High emulsifying activity was obtained in water and saline extracts with corn oil when subjected to heat treatment. This showed the potential of Black gram as good foaming and emulsifying agent. It was observed that the leguminous protein may act as an alternative for egg proteins in food products which need to be studied further.

Key words: Black gram, foaming, emulsification, functional properties.

INTRODUCTION

The storage proteins are of importance in the human diet.Their sole physiological function in the seed is to provide a source of amino acids and nitrogen for seed germination (Kole *et.al.,* 2006). Although seed storage proteins are not biologically active, some storage proteins are known to possess specific metabolic functions in the seed and may be present in sufficient quantities to make significant contributions to seed nutrition.

Despite wide variation in their detailed structures, all seed storage proteins have a number of common properties. They are synthesized at high levels in specific tissues and at certain stages of development. In fact, their synthesis is regulated by nutrition, and they act as a sink for surplus nitrogen. However, most also contain cysteine and methionine, and adequate sulfur is therefore required for their synthesis. Many seeds contain separate groups of storage proteins, some of which are rich in sulfur amino acids and others are poor in them. The presence of these groups may allow the plant to maintain high levels of storage protein synthesis despite variations in sulfur availability.

The strict tissue specificity of seed storage protein synthesis contrasts with that of tuber storage proteins, which may be synthesized in vegetative tissues under unusualconditions (for example, in vitro or after removal of tubers) (AOAC, 1995). A second common property of seed storage proteins is their presence in the mature seed in discrete deposits called protein bodies, whose origin has been the subject of some dispute and may in fact vary both between and within species. Finally, all storage protein fractions are mixtures of components that exhibit polymorphism both within single genotypes and among genotypesof the same species. This polymorphism arises from the presence of multi-gene families and, in some cases, proteolytic processing and glycosylation.

Legume grains occupy an important place in human nutrition, especially for low-income groups of people in developing countries. These are valuable sources of complex carbohydrates, protein and dietary fibre, contribute significant amounts of vitamins and minerals and have high energy value (Sanjeev *et.al.,* 2012). Black gram also known as mash bean belongs to the Leguminosae family. India is the major producer and consumer of black gram with annual production of 1.82 million tons.

Blackgram (*Vignamungo) is* one of the important legumes in the rain-fed farming system in dry and intermediate zones of Sri Lanka. It can be grown under low moisture

and fertility conditions. It has high nutritive value and consists of high content of proteins, vitamins and minerals. Black gram legume reaches 30-100 cm in height. It has a welldiffusely branched from the base. It has sometimes a twining habit and is generally pubescent. The leaves are trifoliate with ovate leaflets and inflorescence is borne at the extremity of a long (up to 18 cm) peduncle and bears yellow, small, papilionaceous flowers. The fruit is a cylindrical, erect pod, and broad. The pod is hairy and has a short hooked beak. It contains 4 mottled seeds (Guadalupe *et.al.*, 1995) adapted to specific environmental conditions.

Dehusked seeds are used as an ingredient in fermented foods like idli, dosa and non-fermented foods like papad (flat biscuits), waries (spicy hollow balls) and cooked dhal. However, it is one of the less-studied legumes. The use of flours as ingredients in food processing is dependent on its functional properties. The functional properties directly or indirectly affect the processing applications, food quality and ultimately their acceptance and utilization in food and food formulations. The functional properties of legume flours are provided by proteins, starch and complex carbohydrates (Salma *et.al.*, 2011).

The objective of the present study was to assess the physicochemical and functional properties of black gram flours from commonly grown locally available cultivars so as to understand their potential applications in making cakes in order to replace the egg. Egg plays an important role in making cakes which enhances the foaming and emulsification properties that result in greater sponginess, increased volume and better texture. Egg consists of albumin which enhances foaming property and globulin which enhances the emulsification property. These proteins are present in black gram as storage proteins.

MATERIALS AND METHODS

The Black gramwith seed coat, Black gram without seed coat and Corn oil were procured from Thiruvanmiyur, Chennai.

REAGENTS

Ethanol (Haymen), Chloroform (SQ), Methanol, Petroleum ether (SQ), Sulphuric acid(SQ)and Whatmann filter paper No.1.

SAMPLE PREPARATION

The seeds of the Black gram (with and without seed coat) were purchased from local market. The seeds were sun dried for 3 h and the dried seeds were ground by using a hammer mill. Finally the flour kept in an air tight plastic container for further investigations.

BACTERIAL STRAINS

Escherichia coli ATCC was isolated from clinical cases and provided by Sri Ramachandra Medical Centre in Porur, Chennaihospital .Bacteria were grown in Nutrient Broth and maintained on nutrient agar slants at 4°C.They were cultured on nutrient broth (Himedia) at 37°C for 24 h.

CHEMICAL ANALYSIS

PROXIMATE COMPOSITION

Samples were analysed for their moisture, ash, fat andprotein (6.25 × N) content according to the standard methods of analysis AOAC.

MOISTURE CONTENT

The moisture content in each flour sample was determined by taking 5g of floursample and drying it in an oven at a temperature of 102 ± 2˚C for 4 hours or to a constant weight. The moisture content was calculated according to the following expression.

$$\text{Percent moisture} = \frac{\text{Weight of sample after drying (g)} - \text{Weight of sample before drying (g)}}{\text{Sample weight (g)}} \times 100$$

ASH CONTENT

The ash content in each flour sample was estimated. Oven-dried samples of 5g were taken in a pre-weighed crucible and charred with a burner. Then it was ignited in a muffle furnace at a temperature of 550-600˚C for 5-6 hours for 3-4 h (2)or till a constant weight of white ash was obtained. The ash was calculated as given below.

$$\text{Percent total minerals} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

TOTAL PROTEIN ANALYSIS

The total protein content of crude black gram extract was determined by Kjeldahl. The Protein was calculated as given below.

$$\text{Percent protein} = \frac{\text{Titre value x Normality of } H_2SO_4 \text{ x } 14.007 \text{x} 6.25}{\text{Sample weight (g)}} \times 100$$

SOXHLET EXTRACTION

100g of sample was taken in Whatmann filter paper No.1 and made into pouch and solvent used is petroleum ether. The thimble was inserted into the Soxhlet extractor. The solvent was filled into the solvent vessel at a temperature of 110-130˚C for 20-30 extraction cycles (4-6 h), depending on the sample nature and the solvent employed. The solvent was dried into a suitable container. Solvent vessel was heated until all of the solvent has been evaporated and condensed in the Soxhlet extractor. Thus the recovered solvent may be reused for subsequent extractions. The vessel containing the fat residue was placed in a drying oven at 103±2˚C and heated to constant weight. Percent crude fat was calculated as follows (AOAC, 1995).

$$\text{Percent crude fat} = \frac{\text{Weight of sample after extraction (g)} - \text{Weight of sample before extraction(g)}}{\text{Weight of sample (g)}} \times 100$$

DEFATTING OF SEEDS BY COLD METHOD OF EXTRACTION

The flour was defatted by extraction with cold acetone for 1 h at 4°C (flour/solvent ratio of 1:2.4 w/v). The slurry was then filtered through Whatman filter paper no.4. Defatted flour was then air-dried, ground and kept in an airtight plastic container for further use (AOAC, 1995).

EXTRACTION METHODS

WATER EXTRACTION

The flour (25g) was extracted by shaking with 100 ml distilled water at 20˚C for 4h (albumin extract) and centrifuged at 3000rpm for 30min (AOAC, 1995).

MSALINE EXTRACTION

The flour (25g) was extracted by shaking with 100ml of 5% NaCl at 20˚C for 4 h(globulin extract) and centrifuged at 3000rpm for 30min. Each extraction was repeated twice (Sai-Ut *et.al.,* 2009).

ETHANOL EXTRACTION

The flour (25g) was extracted by shaking with 100 ml of ethanol at 20˚C for 4h and the slurry was air-dried to evaporate the solvent. Again the dried flour was stirred with 100ml distilled water at 20˚C for 4 h and centrifuged at 3000rpm for 30min (Ju *et.al.,* 2001).

CHLOROFORM EXTRACTION

The flour (25g) was extracted by shaking with 100ml of chloroform at 20˚C for 4h and the slurry was air-dried to evaporate the solvent. Again the dried flour was stirred with 100ml distilled water at 20˚C for 4h and centrifuged at 3000rpm for 30min (Tao *et.al.,* 2001).

ACTIVITY TESTS

FOAMING ACTIVITY

The foaming capacity and stability were calculated for different extracts of ethanol, chloroform, water and saline. In each of the extract different proportions of extract and water have been made. First proportion consists of 25ml of water and 1.5ml of extract. Second proportion consists of 12.5ml of water and 1.5ml of extract. Both are subjected to homogenization. The volume before and after homogenization was noted for foaming capacity. The volume recorded after 60min from the homogenizing time for foaming stability (Sai-Ut *et.al.,* 2009).

$$\text{Foam capacity (\%)} = \frac{\text{Volume after homogenization} - \text{Volume before homogenization}}{\text{Volume before homogenization}} \times 100$$

$$\text{Foam stability (\%)} = \frac{\text{Foam volume after time (t)}}{\text{Initial foam volume}} \times 100$$

FOAMING ACTIVITY OF WATER AND SALINE EXTRACTS AT DIFFERENT TEMPERATURES

The foaming capacity and stability were calculated for extracts of water and saline at different temperatures of -20˚C, 4˚C and 25˚C. 12.5ml of water and 1.5ml of extract were measured. Both were subjected to homogenization. The volume before and after homogenization was noted for foaming capacity. The volume was recorded after 60min from the homogenizing time for foaming stability (Sai-Ut *et.al.,* 2009).

FOAMING ACTIVITY OF FULL FATTED AND DE-FATTED WATER AND SALINE EXTRACTS

The foaming capacity and stability were calculated for full fatted and de-fatted extracts of water and saline (with and without heat treatment (90˚C for 15min)). 12.5ml of water and 1.5ml of extract were measured. Both were subjected to homogenization. The volume before and after homogenization was noted for foaming capacity. The volume was recorded after 60min from the homogenizing time for foaming stability (Tao *et.al.,* 2001).

FOAMING ACTIVITY OF WATER AND SALINE EXTRACTS AT DIFFERENT PH

The foaming capacity and stability were calculated for the extracts of water and saline at different pH 7, 9 and 11 (with and without heat treatment (90˚C for 15min)). 12.5ml of water and 1.5ml of extract were measured. Both were subjected to homogenization. The volume before and after homogenization was noted for foaming capacity. The volume was recorded after 60min from the homogenizing time for foaming stability (Idrees *et.al.,* 2013).

EMULSIFICATION ACTIVITY

The emulsification activity was observed for different extracts of ethanol, chloroform, water and saline. Different compositions of corn oil, water and extracts have been tried for each extract in the ratios of 4:4:2, 2:6:2, 1:7:2, 6:2:2 and 7:1:2. Each composition was mixed up and emulsion was found to form a homogenous solution (13).

EMULSIFICATION ACTIVITY OF FULL FATTED AND DE- FATTED WATER AND SALINE EXTRACTS

The emulsification activity was observed for of full fatted and de-fatted extracts of water and saline (with and without heat treatment (90˚C for 15min). 1ml of extract and 50µl of corn oil were mixed up and emulsion was found to form a homogenous solution (14).

EMULSIFICATION ACTIVITY OF WATER AND SALINE EXTRACTS AT DIFFERENT PH

The emulsification activity was observed for the extracts of water and saline at different pH of 7, 9 and 11 (with and without heat treatment (90˚C for 15min)). 1ml of extract and 50µl of corn oil were mixed up and emulsion was found to form a homogenous solution (15).

ANTIMICROBIAL ACTIVITY

The water and saline extracts were treated against *E.coli*using well diffusion method. The agar plate was swabbed with bacterial culture and the wells were made. 50 and 100µl of water and saline extracts were loaded into the wells and the plate was incubated at 37˚C.

RESULTS AND DISCUSSION

The major component of the legume was found to have protein in the range of 18 to 21%. Moisture content varied from 4.12 to 4.3%, Ash content varied from 3.0 to 3.5%, Lipid content varied from 1.2 to 3%. The proximate composition of legume with and without seed coat tabulated in the following (table1). Black gram (*Vignamungo*) with seed coat having more moisture, ash, protein and fat content rather than Black gram without seed coat. Mostly Black gram consumed as split lentils and the seed coat used as animal feed. But the storage protein such as albumins, globulins are present in maximum amounts in the germ.

Table 1: Proximate Composition

Parameters	Method	With seed coat	Without seed coat
Moisture content (%)	Oven Drying	4.12	4.1
Ash content (%)	Muffle Furnace	3.28	3.0
Crude protein content	Kjeldahl	19.81	18.98
Crude lipid content (%)	Soxhlet	2.2	1.2

FOAMING ACTIVITY OF DIFFERENT EXTRACTS

Water 2 and Chloroform 2 showed higher foam capacity whereas Ethanol 2 and Water 1 showed 100% foam stability. The maximum foam formation was observed in the 2nd composition as given in the Table 4 i.e. higher the protein concentration, higher foam capacity was observed. Ethanol 1 and water 1 extracts showed lower foam capacity. Chloroform 2 and water 2 extracts showed lower foam stability. Ethanol 2 and chloroform 1 showed moderate levels of foam capacity. Ethanol 1, chloroform 1 and saline 1 showed moderate levels of foam stability Table 2, Figure 1. It was previously reported that water extract i.e. albumin protein is responsible for foaming property. Higher the water content showed better foaming capacity whereas higher the saline showed better foaming stability. Normally water above 40% shows higher foaming capacity (16).

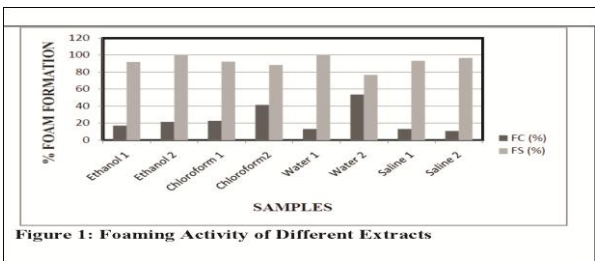

Figure 1: Foaming Activity of Different Extracts

FOAMING ACTIVITY OF WATER AND SALINE EXTRACTS AT DIFFERENT TEMPERATURES

Water and Saline extracts at 25˚C and 4˚C showed higher foam capacity and water and saline extracts maintained at 4˚C and 25˚C showed good foam stability after 1 h. The protein found to show better properties of foaming when it stored at 25˚C and 4˚C. The optimum temperature was found to be 25˚C and 4˚C (Table 3, Figure 2). This study was performed to understand the optimum temperature of the protein which gives foaming property. The extract which was stored at 25˚C showed stable foams. At -20˚C ice crystals breaks the visco-elastic layer of foam.

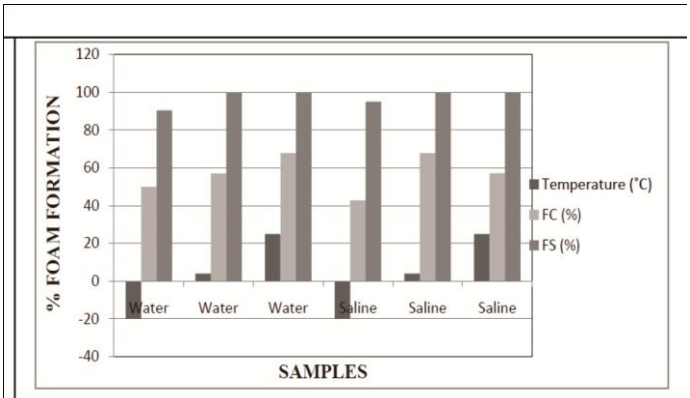

Figure 2: Foaming Activity of Water and Saline Extracts at Different Temperatures

Table 3: Foaming Activity of Water and Saline Extracts at Different Temperatures

s. no	Sample	Temperature (°C)	Foam capacity (%)	Foam stability (%)
1.	Water	-20	50	90
2.	Water	4	57	100
3.	Water	25	67.8	100
4.	Saline	-20	42.8	95
5.	Saline	4	67.8	100
6.	Saline	25	57.14	100

FOAMING ACTIVITY OF FULL FATTED AND DE-FATTED WATER AND SALINE EXTRACTS

Water extract which was full fatted and heat treated exhibited higher foam capacity of 55.4% and saline extract (full fatted and heat treated, defatted) found to have good foam stability (Table 4, Figure 3). This study was extract (full fatted and heat treated, defatted) found to have good foam stability (Table 4, Figure 3). This study was performed to understand role of fat in foaming property. The water and saline extracts which were full fatted showed moderate foam capacity and the extracts which were defatted and heat treated showed lesser foam capacity. The water extract which was full fatted and heat treated showed lesser foam stability. Fat causes detrimental effect for foam formation. Higher the amount of fat, higher the protein.

Table 4: Foaming Activity of Different Water and Saline Extracts

S.no	Sample	Foam capacity (%)	Foam stability (%)
1.	Water (full fatted)	40.4	95.4
2.	Water (full fatted and heat treated)	55.4	68.7
3.	Water (defatted)	40.4	80.1
4.	Water (defatted and heat treated)	23.2	82.6
5.	Saline (full fatted)	44.69	96.2
6.	Saline full fatted and heat treated)	24.33	100
7.	saline (defatted)	40.4	100
8.	Saline (defatted and heat treated)	33.9	96

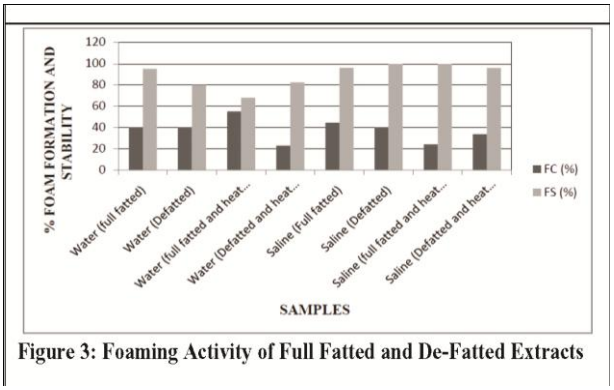

Figure 3: Foaming Activity of Full Fatted and De-Fatted Extracts

FOAMING ACTIVITY OF WATER AND SALINE EXTRACTS AT DIFFERENT PH

Water extract (pH 7 and heat treated, pH 9 and heat treated) showed better foam capacity of 33.97% and saline extract (pH 7, pH 11 and heat treated) found to have good foaming stability (Table 5, Figure 4). This study was conducted to understand the optimum pH. The protein was found to be active at pH 7 and 11. If acidity increases, the foaming stability will be decreased. Some of the factors like acidity, temperature, water content, fat, salt and sugar content influence the foaming properties.

Table 5: Foaming Activity of Water and Saline Extracts at Different pH

S.no	Sample	Form capacity (%)	Foam stability (%)
1.	Water (pH 7)	24.33	81.8
2.	Water (pH 7 and heat treated	33.97	76
3.	Water (pH 9)	23.25	85.2
4.	Water (pH 9 and heal treated)	27.5	84
5.	Water (pH 11)	22.15	87.7
6.	Water (pH 11 and heal treated)	33.97	76
7.	Salute (pH 7)	28.6	100
8.	Salute (pH 7 and heat treated	17.80	95.4
9.	Salute (pH 9)	13.6	99
10.	Salute (pH 9 and heat treated	7.18	95
11.	Salute pH 11	17.89	98.2
12.	Salute (pH 11 and heat treated	7.18	100

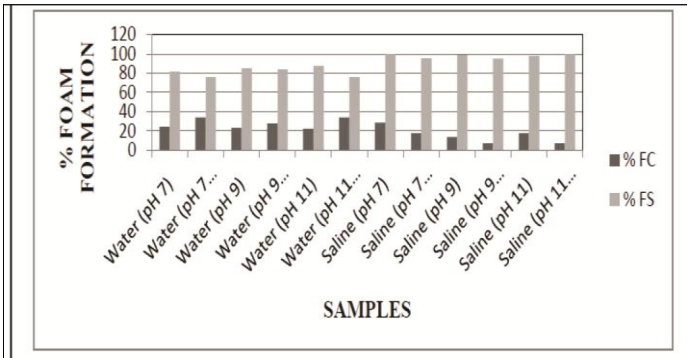

Figure 4: Foaming activity of water and saline extracts at different pH

EMULSIFICATION ACTIVITY OF DIFFERENT EXTRACTS

1st composition = 4:4:2 (oil, water and protein extract), 2nd composition = 2:6:2 (oil, water and protein extract), 3rd composition = 1:7:2 (oil, water and protein extract), 4th composition = 6:2:2 (oil, water and protein extract), 5th composition = 7:1:2 (oil, water and protein extract).

From the above results saline extract of composition 1:7:2 ((oil, water and protein extract) showed the emulsifying property. Sai *et al.,* (2010) reported that the emulsifying properties of the protein isolates were examined as functions of their globulin composition. The effect of the globulins ratio in the sample was probably masked by the presence of phospholipids, which can act as surface active components, as well as by the partial denaturation of the proteins. Higher molecular weight proteins having better emulsification property. Sai *et al.*,(2009) reported that molecular weight of globulin ranges from 95-100kDa. It was found to have better emulsifying property. Concentration of emulsifier is an important factor for enhancing the emulsifying properties (Figure 5).

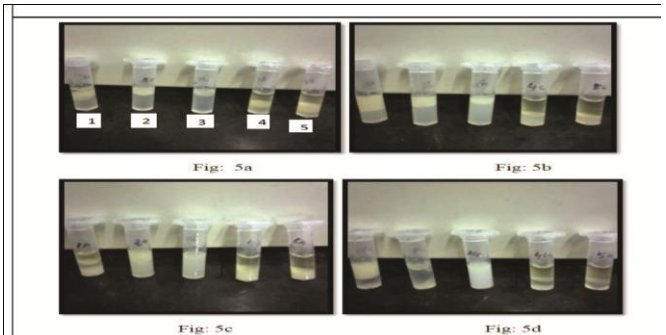

Figure 5: Emulsification Activity Different Extracts
Fig: 5a Emulsion of ethanol extract and corn oil, Fig: 5b Emulsion of chloroform extract and corn oil, Fig: 5c Emulsion of water extract and corn oil, Fig: 5d Emulsion of saline extract and corn oil

EMULSIFICATION ACTIVITY OF FULL FATTED AND DE-FATTED WATER AND SALINE EXTRACTS

The extracts of full fatted and de-fatted water and saline extracts were found to form an emulsion with corn oil after heat treatment. Emulsion is of three types: a) Creaming involves the separation of oil and water layer, b) Flocculation involves the formation of large clusters i.e., partially emulsified, c) Coalescence involves the formation of homogenous solution. At higher temperature oil tends to coalesce and forms homogenous solution (Figure 6, 7).

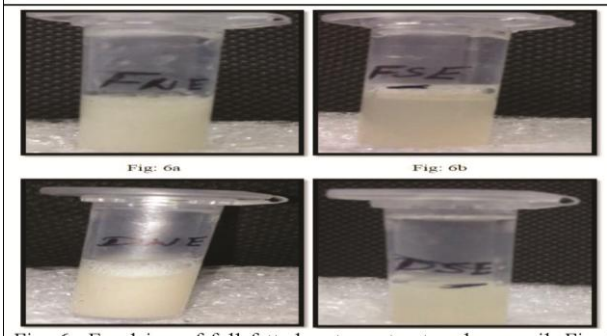

Fig: 6a Fig: 6b

Fig: 6a Emulsion of full fatted water extract and corn oil, Fig: 6b Emulsion of full fatted saline extract and corn oil, Fig: 6c Emulsion of de-fatted water extract and corn oil, Fig: 6d Emulsion of de-fatted saline extract and corn oil

Figure 6: Emulsification Activity of Full Fatted Saline Extracts

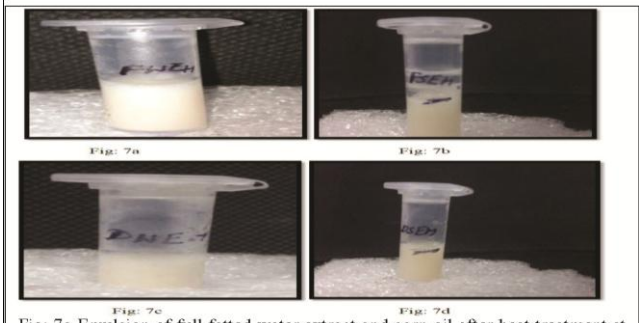

Fig: 7a Fig: 7b

Fig: 7c Fig: 7d

Fig: 7a Emulsion of full fatted water extract and corn oil after heat treatment at 90˚C for 15 min, Fig: 7b Emulsion of full fatted saline extract and corn oil after heat treatment at 90˚C for 15 min, Fig: 7c Emulsion of de-fatted water extract and corn oil after heat treatment at 90˚C for 15 min, Fig: 7d Emulsion of de-fatted saline extract and corn oil after heat treatment at 90˚C for 15 min

Figure 7: Emulsification Activity of De- Fatted Water Saline Extracts

EMULSIFICATION ACTIVITY OF WATER AND SALINE EXTRACTS AT DIFFERENT PH

The water and saline extracts were maintained at different pH of 7, 9 and 11. The extracts without heat treatment were separated into layers of extract and oil after mixing. The extracts which were subjected to heat treatment formed an emulsion as homogenous solution. This study was conducted to understand the optimum pH of the protein. If acidity increases the stable emulsions will not be formed. The stable emulsions will be formed at refrigeration temperature (Figure 8, 9, 10, and 11).

ANTIMICROBIAL ACTIVITY

No inhibition zone was found for the saline and water extracts when treated with *Escherichia coli*. It was conducted to understand the antibacterial activity of the protein.

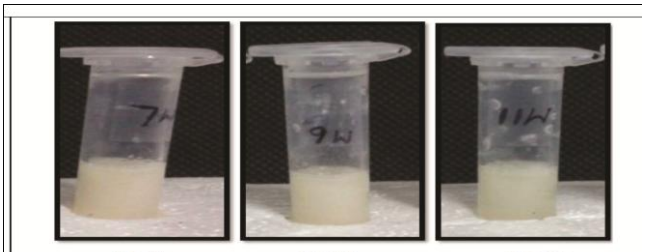

Fig: 8a Fig: 8b Fig: 8c

Fig: 8a Emulsion of water extract at pH 7 and corn oil, Fig: 8b Emulsion of water extract at pH 9 and corn oil, Fig: 8c Emulsion of water extract at pH 11 and corn oil

Figure 8: Emulsification Activity of Water Extracts at Different pH

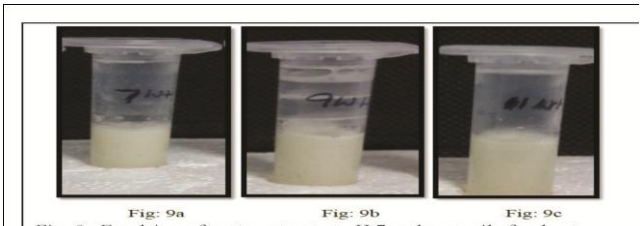

Fig: 9a Fig: 9b Fig: 9c

Fig: 9a Emulsion of water extract at pH 7 and corn oil after heat treatment at 90˚C for 15 min, Fig: 9b Emulsion of water extract at pH 9 and corn oil after heat treatment at 90˚C for 15min, Fig: 9c Emulsion of water extract at pH 11 and corn oil after heat treatment at 90˚C for 15min.

Figure 9: Emulsification Activity of Water and Saline Extracts at Different pH and Temperature

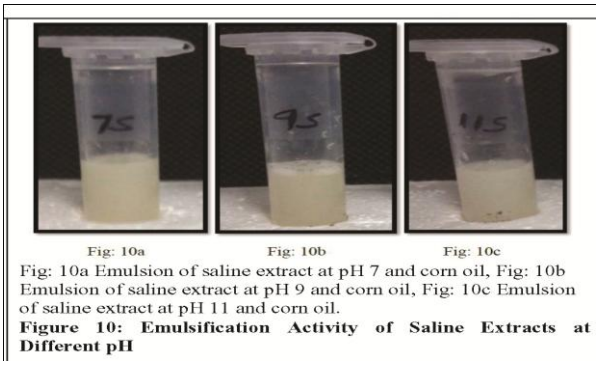

Fig: 10a Fig: 10b Fig: 10c

Fig: 10a Emulsion of saline extract at pH 7 and corn oil, Fig: 10b Emulsion of saline extract at pH 9 and corn oil, Fig: 10c Emulsion of saline extract at pH 11 and corn oil.

Figure 10: Emulsification Activity of Saline Extracts at Different pH

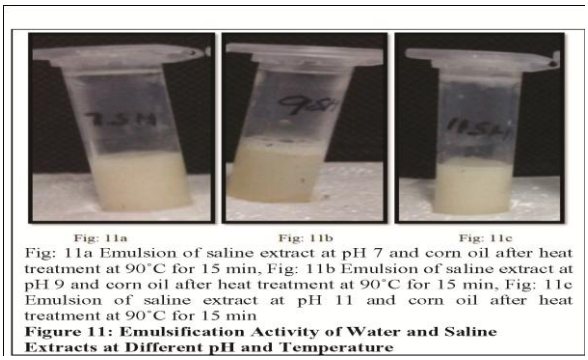

Fig: 11a Fig: 11b Fig: 11c

Fig: 11a Emulsion of saline extract at pH 7 and corn oil after heat treatment at 90˚C for 15 min, Fig: 11b Emulsion of saline extract at pH 9 and corn oil after heat treatment at 90˚C for 15 min, Fig: 11c Emulsion of saline extract at pH 11 and corn oil after heat treatment at 90˚C for 15 min

Figure 11: Emulsification Activity of Water and Saline Extracts at Different pH and Temperature

CONCLUSION

The water extract was found to exhibit better foaming capacity and stability. The water and saline extracts which were maintained at 25˚C were observed to show good foaming capacity and stability. The water extract was found to have more foaming capacity and saline extract was found to have more foaming stability. The water extract at pH 7 exhibited more foaming capacity and saline extract at pH 7 had more foaming stability. Emulsification was observed in the extracts when treated

with corn oil. The homogenous solution was formed when it was subjected to heat treatment.

The future aspect of Black gram (*Vignamungo*) will be used as an ingredient in fermented foods like idli, dosa and non-fermented foods like papad (flat biscuits), waries (spicy hollow balls) and cooked dhal. However, it is one of the less-studied legumes. The use of flours as ingredients in food processing is dependent on its functional properties. The functional properties directly or indirectly affect the processing applications, food quality and ultimately their acceptance and utilization in food and food formulations. The functional properties of legume flours are provided by proteins, starch and complex carbohydrates. The albumin protein dissolves in water and globulin protein dissolves in saline. The moisture, fat, protein and ash was found to be present in higher amounts in the Black gram having seed coat.

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