Optimization for Extracting Iraqi Rice Bran Proteins Cultivar Yasmin and study some of Its Functional Properties

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ABSTRACT

Key words: chemical composition, rice bran, optimal condition for extraction protein, solubility, capacity absorption of Water & Fat, protein emulsion properties, foam properties **Corresponding author:**

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Study of the chemical composition of whole rice bran, and defatted rice bran for Iraqi rice cultivar Yasmine, which was obtained from province of Najaf, showed that the moisture, ash, fat, carbohydrates and protein were 15.8%, 7.9%, 23.5%, 41.1% and 11.7% respectively, for full bran, and 20.9%, 11.2%, 5.5%, 44.25% and 18.15% respectively, for defatted bran. The results of this study showed that the best conditions in the preparation of protein extract from defatted rice bran were by extraction with distilled water after equalized pH to 11 with a ratio bran :water of 1:4 (w : v) for 60 min at agitation speed of 600 rpm.min⁻¹. At these conditions protein percentage were 59.4%. This study revealed that of solubility, emulsifying activity, emulsion stability and foaming capacity and stability of the protein extracted from the defatted rice bran, were highest at pH 11, compared with other pH values with the values of 74.8%, 0.637, 68.8 min and 47% respectively, while the lowest was at pH 4 with the values of 19.3%, 0.057,21.1 min and 7% respectively. The study also showed that the holding capacity of water and fat of the protein concentrate from defatted rice bran was 2.3 ml water . g⁻¹ protein and 2.1 ml oil. g⁻¹ protein respectively.

الظروف المثلى لاستخلاص بروتينات نخالة الرز (Rice bran) العراقي صنف ياسمين ودراسة بعض خصائصه الوظيفية

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الخلاصة

أظهرت نتائج دراسة التركيب الكيميائي لنخالة الرز الكاملة، والنخالة منزوعة الدهن لصنف

الكلمات المفتاحية:

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البربد الإلكتروني:

التركيب الكيميائي، نخالة الرز، الرز العراقي ياسمين التي تم الحصول عليها من قضاء المشخاب في محافظة النجف، ان نسب كل الظروف المثلى لاستخلاص البروتين، من الرطوبة والرماد والدهن والكربوهيدرات والبروتين قد بلغت 15.8% و 7.9% و 23.5% و الذوبانية، قابلية امتصاص الماء 41.1% و 11.7% على التوالي للنخالة الكاملة و 20.9% و 11.2% و 5.5% و 44.25% و والدهن، خواص الاستحلاب، خواص 18.15% على التوالى للمنزوعة الدهن. وبينت الدراسة ان أفضل ظروف تحضير مستخلص بروتين نخالة الرز منزوعة الدهن كانت عن طريق الاستخلاص القلوي عند الرقم الهيدروجيني 11 وبنسبة خلط نخالة : ماء 4:1 (وزن / حجم) ولمدة 60 دقيقة وبسرعة خلط مقدارها 600 دورة / دقيقة والتي اعطت نسبة استخلاص بلغت 59.4%. واظهرت نتائج هذه الدراسة ان قابلية الذوبان وقابلية Ammar.kl20@yahoo.com الاستحلاب وثباتية المستحلب وسعة وثباتية الرغوة للبروتين المستخلص من نخالة الرز منزوعة الدهن الاستلام: 2 / 11 / 2017 كانت اقصاها عند الرقم الهيدروجيني 11 مقارنة مع الارقام الهيدروجينية الاخرى المدروسة وبلغت 74.8% و 0.637 و 68.8 دقيقة و45% على التوالى، في حين كانت أدناها كانت عند الرقم الهيدروجيني 4 بقيم 19.3% و 0.057 و 21.1 دقيقة و7% على التوالي، اما قابلية امتصاص الماء والدهن للبروتين المعزول من نخالة الرز منزوعة الدهن كانت 2.3 مل ماء / غم بروتين و 2.1 مل دهن / غم بروتين على التوالي.

¹ This work is a part of MSc thesis of the 1st researcher

Introduction:

Protein is the main ingredient in many food products. It contributes to the enrichment of nutritional value, flavor as well as many important functional characteristics that imparts to food systems (Giese, 1994). Many different plant proteins have been studied (Sogi *et al.*, 2002; Tomotake *et al.*, 2002; Rangel *et al.*, 2003), Including cereals, which are an important source of food protein for a large population, and many researchers reported that rice protein is better than other grain proteins, such as wheat and corn protein. It is an important ingredient in the preparation of mixtures for infants and children with food allergies (Burks and Helm, 1994), and it caters to the needs of there children aged 2-5 years compared with casein and soy protein isolates (Wang *et al.*, 1999), because it contains the essential amino acids they need, It is also a source of anti-allergic proteins, as well as being rich in lysine. There for , there is expected to be an upward trend in the demand for such affordable economic proteins with an increase demand for food, with the aim of increasing diversity in the production of cheap and nutritious foods, where protein is widely used in many types of food products and contributes to the development of value-added foods or is used as functional ingredients in functional foods, which are currently in high demand.

Rice bran, obtained from rice whitening (which is a secondary product), contains many highnutritive ingrediants, such as proteins, carbohydrates, and many chemical components that have positive effects on human health such as antioxidants such as tocopherol , tocotrienols and gammaoryzanol (Cheruvanky, 2003 ; Chen and Bergman, 2005), In addition, rice bran proteins are characterized by good content of anti-cancer agents (Fabian and Ju, 2011). Rice bran protein, which is extracted from deffated bran, can be added to various foods to increase their nutritional value (Tang *et al.*, 2003a), Thus, rice bran erpresents secondary processing product of raw rice, with high nutritional value, and protein content ranging from 10-16% (Saunders, 1990 ; Hamada, 2000 ; Abdul-Hamid and Luan, 2000), which is higher than any other part of the grain, also rice bran protein is higher in its content of lysine than the endspearm protein or the proteins concentrate from other grain brans (Juliano, 1985), but to date bran is used commercially only to extract edible oil and feed production (Saunders, 1990 ; Tang *et al.*, 2003b ; Parrado *et al.*, 2006).

Rice bran proteins are difficult to separate due to their association with phytic acid and cellulose (Grossman et al.,1980). In addition, their solubility is difficult due to the large number of disulphide bond. Several previous studies have suggested that a number of methods can be used to extract proteins from deffated bran based on the solubility of parts of bran proteins in water, brine, alkaline or weak acid solutions (Tsutsumi *et al.*, 2000). The benefit of rice bran protein, especially as a food ingredient, depends on the properties required to be added to the food (Fabian and Ju,

2011).Functional properties represent the physico-chemical properties of protein, which appear during industrialization, storage or consumption, as well as sensory and nutritional characteristics.

Studies have found that rice bran protein is of high quality and importance in the pharmaceutical and food industries, it can be applied in many food industries such as bread industry (Jiamyangyuen *et al.*, 2005), breakfast cereals, fortification of many protein food products, beverage industry, and can also be incorporated as food ingredients in the meat and sausage industry (Prakash and Ramaswamy, 1996), increasing the water retention capacity of the product and is also good for products that require high water retention (Chandi and Sogi, 2007; Yadav *et al.*, 2011). The absorption of oil is used to increase the sense of oral and retention of flavor. Moreover, high oil-carrying capacity is essential in some product, such as sausage, mayonnaise and sauce salad (Chandi and Sogi, 2007; Khan *et al.*, 2011a,b).

Due to the importance of these wastes and their high nutritional value, in addition to their important functional properties, drew attention to extract proteins from these wastes and estimate these properties in an attempt to introduce them as functional additives in food product, by understanding the functional properties of isolated rice bran proteins, which can be used more widely in food applications to increase consumer acceptance, and because there is no previous study on the functional characteristics of isolating Iraqi rice bran proteins , therefore, this study aimed to

isolate and study some functional properties of rice bran protein concentrate to provide information for the production of bran protein concentrates in an attempt to be used in various food industries.

Materials and methods:

Sample Sources:

The waste of milling (Rice bran (RB)) was obtained from Al- Mishkhab district in Najaf province. It was the result of the process of whitening the rice cultivar Yasmin, which was the product of the year 2015-2016.

Chemical composition of rice bran:

The percentage of moisture, ash, fat and protein was measured by the standard methods in A.O.A.C. (2005). The moisture was estimated by heating at 105 ° C until a constant weight was obtaind. Ash was estimated using mufle furnace at 550 ° C until white ash was obtained. The fat was estimated by using Soxhlelt and hexane as solvent. Nitrogen was measured using kjeldhal apparatus and then multiplied by 5.7 to obtain the protein percentage. Carbohydrate was calculated by the difference. as mentioned by (Pearson, 1970) :

Carbohydrates% = 100 - (Moisture % + Ash% + Fat% + Protein%).

Preparation of defatted rice bran:

The fat was removed from the sample using the cold method. A quantity of rice bran was mixed with hexane at a ratio of (1:3) (w:v), mixed and stirred well, leaving for enough time, then remove of the solvent was discarded by pouring, and add a new amount of solvent, this process was repeated several times until the removal of the largest amount of fat and get a colorless solvent. The deffated sample was spread on aluminum sheet at room temperature in a light layer until flipping dry, then stored in sealed poly ethylene bags at refrigerator temperature until use.

Determination of optimal conditions for protein extraction:

Proteins were extracted from defatted rice bran with distilled water at different pH ranging from 2 - 12, mixing ration of 1:4 (w : v) with continuous stirring for 30 min during extraction, then centrifuged at 15200xg at 25 C for 30 min. Several extraction conditions were also adopted including mixing ratio (1:4, 1:6, 1:8, 1:10, 1:15, 1:20) (w : v), with continuous stirring during the extraction period, then centrifuged at 15200 xg at 25 ° C for 30 min. Several extraction conditions were also adopted, including mixing speed (400, 600, 800, 1200) rpm .min ⁻¹, several times of extraction included (15, 30, 45, 60, 75, 90) min, to follow up the effect of these conditions on the ability of protein extraction after centrifugation the precipitate was neglected. and the supernatant was taken, which represents rice bran proteins. The protein content of the extract was estimated using the biuret method (Plummer \cdot 1988) and the protein extraction precentage was calculated according to the following equation (Betschart *et al.*, 1977) :

Protein extraction % = (protein ratio in supernatant / protein ratio in bran) \times 100

The bran proteins were extracted using the best conditions selected from the previous points which gave the highest percentage of protein. The protein extract then dried in an electric oven at 40 - 45 C to produce the protein powder, which stored in sealed poly ethylene bags at refrigerator temperature until use in studying of functional properties.

Functional properties:

The functional properties estimated of Yasmine rice bran protein concentrate and Casein as a comparative sample .

Determination of solubility:

The solubility of bran proteins and casein was estimated as mentioned by Gnanasambandam and Hettiarachchy (1995) at different pH values ranging from (2-12) then the

protein was estimated using the biuret method (Plummer (1988)). The solubility of the protein was calculated using the following equation:

Solubility % = (protein ratio in supernatant / protein ratio in 100 g protein) \times 100

Holding capacity of water and fat:

The holding capacity of water and fat for rice bran protein concentrate were determined according to Aloys and Zhou (2006), by placing 1 g of protein concentrate with 5 ml distilled water or sunflower oil in 10 mL graduated centrifuge tubes. The content were mixed well with a glass rod and left for 30 minutes at room temperature, then centrifuged at 3000xg for 10 min, the volume of non-absorbent water and fat was recorded by receiving it in a cylinder. The absorbed volume was calculated and expressed as ml water / g protein for water and ml oil / g protein for fat.

Emulsifying properties:

The emulsifying properties of rice bran protein concentrate were estimated according to Pearce and Kinsella (1978), by mixing 20 ml of sunflower oil with 36 mL of 1% solution protein (w : v) at different pH ranging (2-12). The mixture were mixed and homogenized at 8000xg for 1 min at room temperature, then 50 µl of emulsion was taken from the bottom of the container will taken immediately after homogenization and mixed with 5 ml of 0.1% sodium dodecyl sulphate (SDS) solution, and the absorbance of emulsions was measured directly at 500 nm and this was considered as emulsion capacity. To determine the stability of the emulsion, the emulsion formed leaved at 25 ° C for 10 min, then take 1 ml of emulsion and mix with 5 ml of 0.1% SDS and measured the absorbance at 500 nm and calculate the stability according to the following equation:

stability of the emulsion = $\frac{A \circ \times t}{A1}$ A \circ = Absorbance at 0 min , T = time (10) min , A1= is the change in absorbance occurring over the interval time.

Foaming capacity and stability:

The foam capacity and stability of the protein concentrate was estimated according to Kato et al. (1983), by dis solving 1 g of protein concentrate in 100 ml of distilled water at different pH values ranging (2-12), the volume of mixture was determined and after mixing using the homogenizer at 10000xg for 1 min, the volume of mixture was also determined and the foam capacity is calculated according to the following law:-

Foam capacity % =
$$\frac{volume \ after \ mixing \ (ml) - volume \ before \ mixing \ (ml)}{The \ volume \ of \ the \ solution \ before \ mixing}$$

The foam stability was determined by followed the formed foam at different intervals (0, 15, 30) min, and calculated according to the following law:-

Foam stability $\% = \frac{volume \ of \ foam \ after \ (time)}{The \ volume \ of \ the \ first \ foam} \times 100$

Results and discussions:

Chemical composition of rice bran:

Table (1) illustrates the chemical composition of whole and defatted rice bran, and it shows that the percentages of moisture, ash, fat and carbohydrates are 15.8%, 7.9%, 23.5% and 41.1% respectively for full bran and 20.9%, 11.2%, 5.5% and 44.25%, respectively, for defatted rice bran. This result may be agreed with some previous studies in the same field and may be different with others. Sanchez et al. (2004) reported that moisture content was 12%, ash 7-8% and carbohydrates 52.3-43.1%. Rosniyana *et al.* (2009) found that the fat ratio was 25.5 - 30.4% and the carbohydrates was 25.9 - 47.4%, while Ambreen *et al.* (2006) pointed out that the ash ratio was 10.6%., Kaur *et al.* (2011) reported that the fat was 19.31%, while ash and carbohydrate were 6.72% and 36.6% respectively for whole bran, but they were 3.44%, 8.46, and 50.80 for fat, ash and carbohydrate respectively of defatted rice bran, Abdul-Hamid *et al.* (2007) found that the moisture content and ash were 8.5 - 12.6% and 7.7% respectively for defatted rice bran, Yadav *et al.* (2010) reported that the ratio of fat and ash was 3.5% and 12.5%, respectively and carbohydrates was 50.7% of defatted rice bran. Rosniyana *et al.* (2009) reported that carbohydrate was 25.91 - 47.14%.

Component% Bran	Moisture	Ash	Fat	Carbohydrate	Protein
full	15.8	7.9	23.5	41.1	11.7
Defatted	20.9	11.2	5.5	44.25	18.15

 Table (1) The chemical composition of the full and defatted rice bran cultivar Yasmin

The protein, which was the main objective of this study, is shown clearly in the same table that it was 11.7% in the whole bran and 18.15 in the defatted bran, this demonstrates the efficiency of removal of fat in increasing the protein concentration in the defatted sample. The percentage of protein reached in this study may be differ different from some previous studies, Roy and Chandra (2005) and Peirce and Hammond (2013) pointed that the proportion of full bran protein extracted by heat processing ranged between 11.3 - 14.9 and 10.6 - 13.4%, respectively, Singh *et al.*, (2013) reported that the protein content of defatted bran was 13%, Khan *et al.* (2009) and Faria *et al.* (2012) reported that the protein content of defatted bran was 18.16 - 19.05% and 18.93 - 19.38% respectively, Abdul-Hamid *et al.* (2007) reported that the protein content of defatted bran was 8.8 - 15.2%. The difference in results may be due to the diverse in the variety of rice used as well as the conditions of agriculture and fertilization, in addition to the bleaching conditions used, as reported by Rao and Reddy (1986) whose indicated the effect of bleaching time and pressure used.

Optimal conditions for protein extraction:

Effect of extraction conditions on the ratio of extracted proteins:

Figure (1) shows the effect of pH on the percentage of protein extraction from deffated rice bran. As shown that there was an increase in the percentage of protein extraction by increasing the pH towards the basal numbers, where the pH 11 gave the highest percentage reached 44.5%, then returned slightly decreased at the number 12 to 43.8% compared to other pH values. These results are consistent with what recorded by both Guppta *et al.* (2008) and Fabian and Ju (2011) that the ratio of protein extraction increases with the rise of pH towards basal numbers, they pointed to an extraction rate of 30-80% at pH 7-12 ,%, but it was less than what they reached, Gnanasambandam and Hettiararchchy (1995) found that the extraction ratio at pH 11 was the best since the protein content obtained was 71-73%. While this result was different from what obtained by Theerakulkait *et al.* (2006) who reported that the best pH for the extraction is 9.5 and which He did not differ significantly from the pH 10, but was significantly higher than the extraction rate of about 50% and then decreased to 46% at pH 10.

The solubility of the protein usually increases when moving away from the isoelectric point because the charge of the proteins at this point is neutral. In the rise or fall of this pH value the solubility increases, because the protein carries a positive or negative charge and the net charge of the protein was increased as a result of the conversion of amino acids to the ionized form, and thus the protein solubility increases, since most food proteins are acidic proteins, therefore they had low solubility in acidic pH values ranging from 4 to 5, and this increases with high pH's towards the base, Attia *et al.* (2000) reported that the solubility of the protein increases at more than pH 7.5, and many studies have shown an increase in the solubility of the protein in the high acid or base pH values, while Ogunwolu *et al.* (2009) and Mao and Hua (2012) reported that the solubility of most proteins is similar and it was at lower level at pH 4 - 5 and increase by increasing the pH by moving away from isoelectric point.



Figure (1) The effect of pH on the protein extraction from Yasmin defatted rice bran

Figure (2) illustrates the effect of the mixing ratio on the protein extraction of the defatted rice bran. As shows that the ratio 1:4 gave the highest extract rate of 44.5%, then it was decreased to 38% at 1:6, the other mixing ratios gave 28.5%, 29.7%, 26.8%, and 22.4% for 1:8, 1:10, 1:15 and 1:20 respectively. This result is consistent with what was indicated by Theerakulkait *et al.*, (2006) that the best ratio of extraction was 1:4 (w / v), but do not agreed with Naji (2016) who reported that the best extraction ratio was 1:6 when studying Mashahab-2 rice bran. It may be attributed to the fact that the increase in the mixing ratio caused dilution and difficulty in extracting, especially that the layer of amino acids associated with the protein has a complex layer in terms of composition and precipitation.



Figure (2) The effect of the mixing ratio on the protein extraction of Yasmin defatted rice bran

Figure (3) shows the effect of the time on extracting of the protein from defatted rice bran. It was found that the best extraction was at 60 min, it also shows an increase in the percentage of

protein with increasing extraction time, then it was decreased gradually. The extraction rate was 52.5% at 60 min, then it was decreased to 41.8% at 75 min, this result was consistent with Naji (2016) that reported the best time to extract Mashahab-2 rice bran protein was 60 min, while it was not consistent with Theerakulkait *et al.* (2006). which indicated that the best time for extraction was 45 min, because there was no significant increases in the percentage of protein extracted at time increase. This might be due to the poor solubility of some portions of protein with an extensive disulfide bonding and aggregation, and the protein in rice bran are a complex mixture and could bind with other compounds in rice bran such as phytate, fiber (Hamada, 1997).



Figure (3) The effect of extraction time on protein extraction of Yasmin defatted rice bran

Figure (4) indicates the effect of the mixing speed on the protein extraction of defatted rice bran, as shown there is an inconsistent relationship between the protein extraction rate and the mixing speed, where the maximum value of 57.5% at the mixing speed 600 rpm compared with the others, and decreased back at the highest mixing speed, this decreasing may be due to the denaturation of a protein at a high speed. This result is consistent with Naji (2016) that reported that highest rate of extraction of Mashahab-2 protein was at a speed of 600 rpm . min ⁻¹, while it inconsistent with the Theerakulkait *et al.* (2006) who found that the best mixing speed was 500 rpm . min ⁻¹, although the extraction rate was up at a higher speed, but at a small and insignificant rate.



Figure (4) The effect of mixing speed in protein extraction of Yasmin defatted rice bran

The protein was extracted to study some of its functional properties by using the optimum conditions determined, so it extracted, with pH 11, at a mixing ratio of 1:4 (w/v) for 60 min and 600 rpm . min ⁻¹. These extraction conditions gave a percentage rate of 57.5%, which was close to Naji (2016) (53%) and higher than Theerakulkait *et al.* (2006) (44.4%), Hamada (1997) reported that the extracted protein is not high, and may be due to a poor solubility and the fact that some parts of the protein were not dissolve completely, with an extensive disulfide bonding and aggregation. Betschart *et al.*, (1977), Juliano (1985) and Hamada (1997) reported that rice bran protein is a complex mixture because it can be associated with other compounds completely such as fiber and phytate, Khan *et al.* (2009) found that the protein ratio will differ according to the treatment methods used, and reborted that untreated bran was 78.9%, but treated bran using microwave and heat, were 69.61% and 67.59% respectively, Zhang *et al.* (2012) reached an extraction rate of 32.9% and 44.79% when using two extraction methods including basal extraction and alcalase enzyme, respectively.

Functional properties of rice bran protein isolate: Protein solubility:

The solubility of rice bran proteins concentrate and casein was estimated at different pH (Figure 5), the solubility at 11 and 4 gave higher and lower solubility of 74.8% and 19.3% respectively, While the highest and lowest solubility of Casein was at pH 12 and 4 at 81% and 41, respectively. This result is consistent with Zhang *et al.* (2012) that the protein solubility was 72.5% and 84.56% at pH 11, when using two extraction methods including alkaline extraction and Alcalase enzyme, respectively.



Figure (5) The solubility of Yasmin defatted rice bran protein concentrate at different pH values

As seen from the figure, the solubility increases with the increase of pH towards the base. The highest solubility of defatted rice bran proteins at pH 11 may be due to the negative charge of protein molecules at this pH, which increases the force repulsing, as well as increased interactions with water Zayas (1997), however, solubility at pH 11 is not high, and this can be due to protein interactions or association with other compounds extracted from rice bran with protein (Juliano, 1985; Hamada1997). The solubility at pH 2 is higher than pH 4, as shown by the same figure, this shows that the iso electric point of the protein is close to the pH 4, which is consistent with Bera and Mukherjee (1989), Gnanasambandam and Hettiararchchy (1995) and Wang *et al.* (2014).

The solubility of proteins depends on several factors including pH, composition and sequence of amino acids, interactions with other components, presence of salts (Ivanova *et al.*, 2013), the increase of solubility with the increase or decrease of pH is due to the increase of the net charge of the protein and the conversion of amino acids to the ionized form. Several studies have

indicated increasing solubility with increasing pH to acidity and alkalinity away from iso electric point (Ge *et al.*, 2000).

Water and fat absorption capacity:

Figure (6) shows that water absorption capacity of rice bran protein isolate was higher than the fat absorption capacity, it was 2.3 ml water and 2.1 ml oil . g⁻¹ protein respectively. This is similar to the comparative sample as the water absorption capacity is higher than the fat absorption capacity. This result was consistent with some previous studies, Aletor *et al.* (2002) reported that the average water and fat holding capacity was 2.66 and 3.7 ml . g⁻¹ for Basmati 370, respectively, Cheng *et al.* (2009) found that the fat binding ratio ranged 2.4 - 3.3 ml . g⁻¹, Gupta *et al.* (2008) reported that water and fat uptake ranged 1.02 - 2.27 and 1.64 - 6.89 ml . g⁻¹ respectively, Chandi and Sogi (2007) reported that the water and fat absorption capacity ranged 2.48 - 3.77 and 3.74 - 9.18 ml . g⁻¹ respectively for heat process protein.



Figure (6) Water absorption and fat capacity of Yasmin defatted rice bran protein concentrate

Aletor et al. (2002) reported that the water absorption capacity may be due to the distribution of polar and non-polar groups on the surface of the protein fraction and these aggregates are able to bind to water. Proteins that have high water absorption capacity can help reduce moisture from packaged baked goods as well as to preserve the freshness and moisture of the oral taste of baked goods. Rice bran protein has a good water absorption capacity and can be used in products that require high water retention. The high absorption of water results from the presence of polar amino acids in the structure of the protein, and decrease in water absorption is largely due to changes in protein.

Several studies have reported the effect of several factors on the water absorption of protein, including the size of the protein molecule that affect the amount of water that is confined to the protein. The degree of binding of proteins to each other, as well as hydrophilic groups (polar groups) aggregates in the protein molecule (Aurelia et al., 2009), the chemical composition of protein and its association with other compounds such as sugars, fat, tananate and others (Han and Khan, 1990).

Non-polar side chains increase fat absorption capacity by binding hydrocarbon chains of fat together (Khan et al 2011b). Fat absorption is due to the distribution of polar and non-polar groups on the molecular surface of the protein these groups are able to bind and retain fat. The protein's ability to bind to fat is due to the mechanics of binding with the non-polar side chains of the protein, which is related to the chains hydrocarbons and thus hold fat inside the protein molecule because the protein contains the non-covalent bonds such as electrostatic forces and hydrophobic forces. Thus, it increases the protein's absorption of fat (Lin and Zayas, 1987).

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Emulsifying activity and emulsifying stability:

Figure (7) shows that there was an increase in the activity of emulsion formation with increased pH values, at pH 11 the protein concentrate had the highest of emulsifying activity was 0.637 (at 500 nm) which was higher than the casein of 0.54, while the lowest emulsifying activity at pH 4 was 0.057, Zhang *et al.* (2012) reported that the emulsification of rice bran protein concentrate was 0.634 when Alcalase was used in extraction, while Esmaeili *et al.* (2015), using two types of rice, Shiroodi and Tarom, and found that the highest activity of emulsion formation was 0.165 and 0.12 (at 500 nm), respectively at pH 8. Damodaran (1996) reported that the highest stability of the emulsion at pH 9 was 0.167 and the lowest stability of emulsifier at pH 4 was 0.063 (at 500 nm).



Figure (7) The Emulsifying activity of the Yasmin rice bran protein concentrate at different pH

Figure (8) shows the emulsion stability of the rice bran protein at different pH values. As indicated that emulsion stability increased with increasing pH, as at pH 11 the protein gave the highest stability within 68.8 min and it was decreased to 56 min at pH 10, Zhang *et al.* (2012) reported that emulsion stability was 24.26, 25.96 min when using two methods for extraction protein including basal extraction and alcalase respectively, but Esmaeili *et al.* (2015) when use two types of rice, Shirodi and Tarom, found that the emulsion stability was highest at pH 8 which was 68 and 125 min respectively, Damodaran (1996) reported that the emulsion stability at pH 9 was 43.15 min.

The properties of emulsion are associated strongly with the properties of hydophoic protein surface and protein solubility (Damodaran, 1996). The presence of a large number of small oil droplets in the emulsion is explained by the high absorption value at 500 nm (Pearce and Kinsella, 1978). The high emulsion activity and stability in basal pH and it's decrease in acidic pH is due to the high protein solubility in basel pH (Mangino, 1994), Bandyopadhyay *et al.* (2008) reported that emulsifying is mainly based on the diffusion of peptide bonds on the water and oil interface, Wanger *et al.* (2000) reported that small hydrophilic peptides have weak emulsifying properties due to their weak ability to reduce surface tension, Esmaeilia *et al.* (2015) reported that alcalase use not only affects solubility but improves the emulsifying properties of extracted proteins, and heat treatment during extraction oil causes the protein denaturation extracted from rice bran, which leads to the increase of groups hydrophobic and therefore affect the functional properties.



Figure (8) Emulsion stability of Yasmin rice bran protein concentrate at different pH

Foaming capacity and stability:

Figure (9) shows that the capacity of foam formation of rice bran protein concentrate increase with increased pH. At pH 11 the protein concentrate had the highest capacity of foam formation which was 45%, while the lowest capacity was at pH 4 with 7%, while the foam capacity of the casein was higher in all pH. Sirapat *et al.* (2014) reported that the foam capacity of the protein concentrate was higher when compared with the raw rice bran, and the foaming capacity of the protein isolate extracted using basal was 57.5%, Yadav *et al.* (2011) reported that the foam capacity of the rice bran protein concentrate by basal methods was 11.0%. Chittapalo and Noomhorm (2009) found that the foaming capacity of the rice bran protein isolated from rice bran ranged between 20-92%, and the average of foam capacity at pH 5 at 30 and 45 ° C was 20 and 30%, respectively.

The foam capacity and stability require different molecular properties, foam capacity requires quick protein uptake at the air and water interface during mixing, and quick response to change, matching and rearrangement on the interface, and it depends mainly on its solubility and the protein must be stable in the water layer Were *et al.*, (1997). Lawal (2004) suggests that increased foam formation with increasing pH is due to an increase in the electrical charge of the protein and thus increasing solubility and protein elasticity, and this leads to protein spreading at the water-air interface, surrounding the air bubbles and thus increasing the formation of the foam.

Figure (10) illustrate the foam stability of the rice bran protein concentrate at different pH values. As that foam stability increased with increasing pH, at pH 11 the protein gave had the highest stability while maintaining this stability even after 30 minutes.

Chandi and Sogi (2007) reported that protein of basmati 370 stability was weak at pH 9 which was 4-5 sec, while foam stability of Basmati 386 was more stable at the same pH, Meuser *et al.* (2001) reported the importance of pH control and its significant impact on the volume and stability of foam, Gupta *et al.* (2008) noted that the foam stability of the protein extracted from rice bran at 60 ° C was weak at pH 7 which lasted for 11 - 15 min, while stability at 30 ° C and the same pH was 180 ° C they also stated that the foam stability decreases when the temperature increases more than 30 ° C at pH 7 and 9. Esmaeili *et al.* (2015) noted that the foam stability of the Shiroodi and Tarom rice bran protein at pH 5 was 36 and 50 minutes respectively. At pH 7, the foam stability was 32 and 33 minutes respectively, then decreased to 32 and 38 minutes at pH 8.

The foam stability of the protein concentrate of rice bran is good and similar to egg white due to the lack of secondary and tertiary structure in the protein molecule, both Halling (1981) and Damodaran (1997) reported that the protein isolated from rice bran by phytase and xylanase gives more flexibility to the random structure, due to loss of secondary and tertiary complex structure due to the loss of minerals, cellular parts and phytate.



Figure (9) Effect of pH in the foam capacity of Yasmin rice bran protein concentrate



Figure (10 A) Effect of pH in foam stability of rice bran protein concentrate after 1 min



Figure (10 B) Effect of pH in foam stability of rice bran protein concentrate after 15 min



Figure (10 C) Effect of pH in foam stability of rice bran protein concentrate after 30 min

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