

Full Length Research Paper

Impact of electric voltages on the emulsification capabilities of okra seed protein-rich extract

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A portion of okra seed protein-rich extract (PRE) prepared with NaCl solution (5 g/L) was hydrolyzed at 110°C for 5 h (1 atm. pressure). The PRE (sample A), hydrolyzed PRE-B, whole okra seed flour-C and gelatin-D were tested for emulsification capacity (EMC) and emulsion stability (ES). The emulsions were prepared and tested in (i) normal environmental condition (o/w and w/o), (ii) current carrying beaker-CB, (iii) Ohmic heating beaker-OB. The tests were carried out at zero to 240 V. The results showed that at 200 V, the EMC were 85.71, 80.50, 88.23, 85.31, 94.71, 94.70, 90.68, and 83.3% for A_{CB}, A_{OB}, B_{CB}, B_{OB}, C_{CB}, C_{OB}, D_{CB}, and D_{OB}, respectively. The respective ES are 23, 31, 25, 43, 98, 61, 98, and 65%. At 200 V, okra product C_{CB} had the highest EMS and ES (94 and 98%, respectively). At 95% oil (w/o) emulsion, EMC for PRE and gelatin were 6.0 and 1.9%, respectively. At 5% oil (o/w) emulsion, the corresponding EMS and ES is 84 and 94%. Hydrolyzed okra seed PRE-C_{CB} is possibly a hydrophilic emulsifier, combining high (94%) EMC with high (98%) ES.

Key words: Okra seed, protein-rich extract, hydrolysis, emulsification, electric field.

INTRODUCTION

The amino acid composition of okra seed was found to be similar to that of soybean, yet the protein efficiency ratio (PER) was higher for okra seed (Karakoltsidis et al., 1975; Martin et al., 2006). These researchers also showed that the PER of okra seed flour, heated at 130°C for 3 h was not different from non-heated flour.

According to Vickie and Elizabeth (2008), the best

emulsifiers are proteins, which uncoil or denature and adsorb at the interface and interact to form a stable interfacial film. Proteins tend to uncoil such that their hydrophobic sections are oriented in oil and hydrophilic sections are oriented in water. Hence, a series of loops, trains and tails may be envisioned at the interface. This confers emulsification capabilities on proteins. Vegetable

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Figure 1. Production of PRE (Gilles, 2002).

proteins are commonly used as emulsifiers in food processing, perhaps due to the high molecular weights, which enables them to stabilize the emulsion, by elevation of the systems' viscosity when gelled (Vickie, 2008). Okra seed protein, with all its quality attributes can attract more utility to the crop, if the seed protein can be fashioned out as a major emulsifier in the food industry. Makers of cakes particularly at homes, may want to avoid much of egg yolk where there is need to avoid cholesterol in the meal. A vegetable protein from okra with appreciable emulsification capabilities will be helpful in such situations.

The objectives of this work are to obtain protein-rich extract (PRE) from okra seed, hydrolyze the PRE and employ the PRE, hydrolyzed PRE and whole okra seed flour in the production of emulsions at normal environmental conditions as well as under the influence

of electricity. Gelatin is included as a standard.

The aim is to record particularly, the positive impacts of electric voltages on the emulsification capabilities of okra seed products measures that can increase the usefulness of okra.

MATERIALS AND METHODS

Preparation of PRE

Okra seed was soaked in water for 24 h, allowed to drain for 8 h before milling using an attrition mill. The hull was then sieved out using 0.2 mm sieve. Oil from the whole okra seed flour was extracted using hexane at 45°C. Three successive extractions were done. The oil-free flour (100 g) was added into 1 L of NaCl solution (5 g/L).

The mixture was stirred for 15 min. Extraction was allowed to go on for 6 h. The solution was then centrifuged at 2000 rpm for 30 min. The pH of the supernatant was adjusted to 4.0 with acetic buffer tablet. After 15 min of stirring, the supernatant was centrifuged at 2000 rpm for 30 min. The solid residue-PRE was dried in a cabinet dryer at 60°C (Figure 1).

Hydrolysis of protein rich okra seed extract

The slurry of the PRE was prepared by weighing 10 g of the extract into 250 ml beaker; 100 ml of distilled water was added and mixed properly with a glass stirrer, until the PRE was completely suspended in the water. 5M HCl (200 ml) was added to the mixture and the pH was monitored until it dropped to 2. The acid was used as catalyst in hydrolyzing the protein. The hydrolysis was completed in an autoclave by the use of steam at temperature of 121°C for 5 h. At the end, the sample was mixed with 20 ml of 3M NaOH, increasing the pH of the hydrolyzate to 6 (Gilles, 2006; Balami, 2004).

Determination of emulsification capacities (EMC)

Emulsions were prepared with each of these as emulsifiers-whole okra seed flour, PRE, hydrolyzed PRE and gelatin. Each of these (2 g) was mixed in 100 ml of distilled water and 10 ml of dried groundnut oil and homogenized for 5 min using a PROLABO homogenizer (silver model L2R) at 2500 rpm. The emulsion was then poured into graduated centrifugal tube and centrifuged at the speed of 2500 rpm for 15 min.

EMC (%) is given by:

$$\frac{\text{Length of emulsified layer}}{\text{Length of whole content of tube}} \times \frac{100}{1}$$

One of the factors that affect the EMC of proteins according to Riken (2002) is the extent of conformational arrangement at the interface of oil and water, an attribute of solubility of the protein. For this reason, a sample of the PRE was hydrolyzed at pH 2 for 5 h using an autoclave at 1 atm. EMC of the hydrolyzed sample was assayed alongside others as described earlier (Balsam, 1994).

Conformational change can also be brought about by ionization, presence of charges and charged particles (Balsam, 1994). Hence, the research design varied conditions of emulsification. Emulsions were prepared: (1) in an Ohmic heating beaker-OB (Figure 3) and (2) in a current carrying or conducting beaker-CB (Figure 2). Ohmic

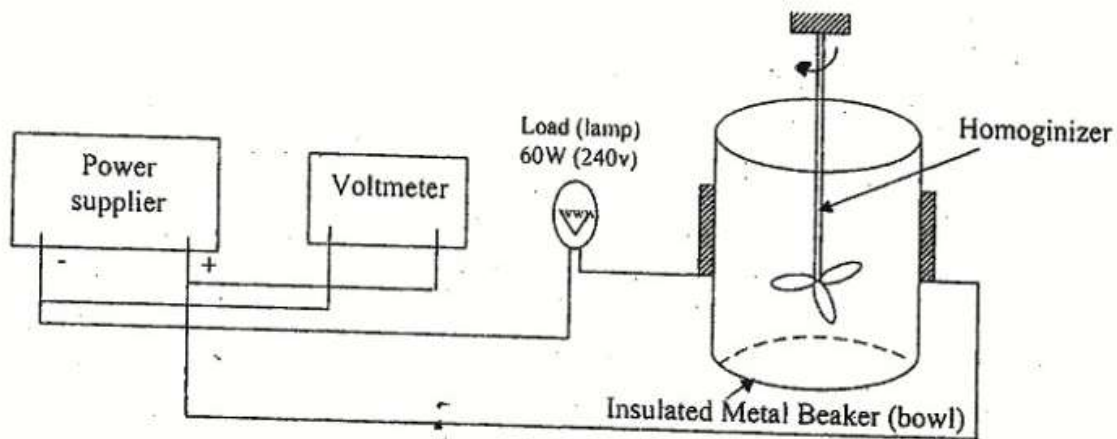


Figure 2. Diagram illustrating the emulsification of samples in a current carrying conductor in an electric field.

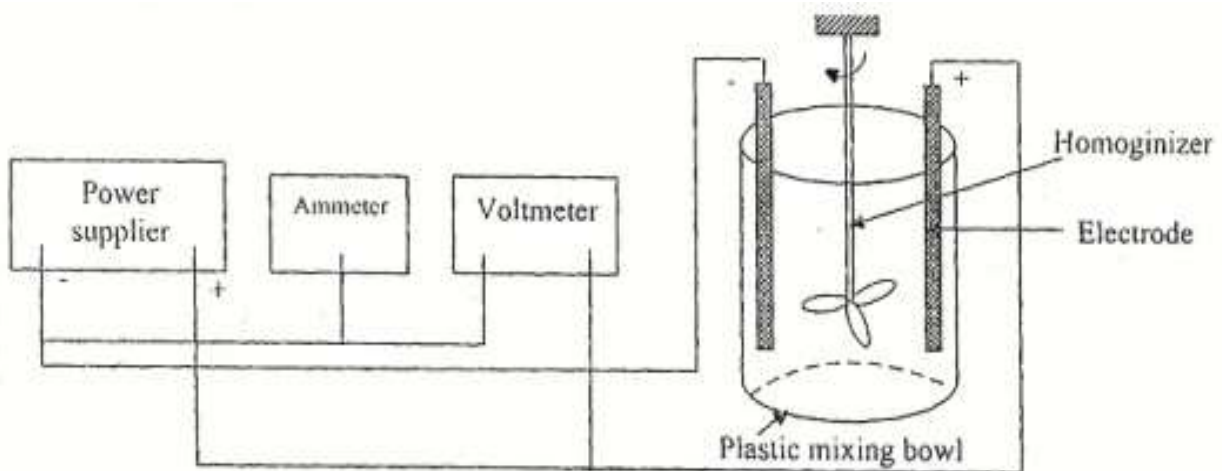


Figure 3. Diagram illustrating the emulsification of samples in an Ohmic heating beaker.

heating beaker permits the flow of free ions to the poles while a current carrying conductor allows the flow of electrons or electricity through the walls of the reaction beaker (Figure 2). Finally, emulsions were prepared with oil concentration of between 5 and 95%, at normal atmospheric conditions, using PRE (in one batch) and gelatin (in another). The EMC were measured and compared.

Emulsion stability (ES)

After reading off and calculating the EMC, the marked tubes, containing the centrifuged samples were stored in racks and kept on the shelf for 5 days. ES (%) was calculated as (Balami, 2004; Gilles, 2006):

Height of remaining emulsified layer / Height of the whole column \times 100

RESULTS AND DISCUSSION

EMC of okra seed products at different voltages of electricity

There is significant difference in EMC among samples both of current carrying beaker (CB) and Ohmic heating beaker (OB) at zero voltage. The hydrolyzed PRE (94.7%) is significantly higher than the rest. It is higher than gelatin (84.85%), a common food emulsifier which in this study served as a control (Cole, 2000). Changes in particle size should be a major consequence of conversion of the whole okra seed into PRE and subsequently to hydrolyzed PRE. The larger the protein molecule, particularly the globular protein, the smaller the

Table 1. Emulsification capacities (%) at different voltages of electricity in CB and OB.

Sample	Emulsification capacities (%)					
	0	200	210	220	230	240
A _{CB}	79.14 ^a	85.71 ^b	88.51 ^e	87. ^{94f}	84.09 ^c	85.23 ^c
A _{OB}	79.14 ^a	80.50 ^c	81.01 ^f	81.21 ^c	82.42 ^c	83.33 ^c
B _{CB}	89.24 ^a	88.23 ^b	88.0 ^e	85.36 ^f	82.94 ^c	80.55 ^c
B _{OB}	89.24 ^a	85.31 ^b	80.12 ^f	78.03 ^c	78.03 ^c	78.02 ^c
C _{OB}	94.74 ^b	94.65 ^d	94.71 ^g	95.56 ^g	95.76 ^b	96.19 ^d
C _{CB}	94.74 ^b	94.71 ^d	94.70 ^g	94.70 ^g	94.51 ^b	88.76 ^d
D _{OB}	84.85 ^a	89.46 ^b	90.68 ^g	94.68 ^g	94.80 ^b	95.70 ^d
D _{CB}	84.85 ^a	84.61 ^c	83.31 ^f	83.01 ^c	82.91 ^c	82.88 ^c

A: Whole okra flour; B: protein rich extract; C: hydrolyzed protein rich extract; D: gelatin; CB: current carrying beaker; OB: Ohmic heating beaker; Sample on same column with same subscripts are not significantly different ($p \leq 0.05$)

solubility. The increase in EMC, for PRE, between 0 and 240 V is 96.19 to 94.74%. The highest increase was exhibited by gelatin (95.0 to 84.85). PRE under increasing voltage, showed rather decreasing EMC.

The impact of increasing voltages (200 to 230) for the hydrolyzed PRE, is increased EMC (94.65 to 95.76) for CB and decreased EMC for OB (94.71 to 94.51)

EMC in a current carrying conductor-CB, for all the samples, kept increasing as the voltage increased. The only exception to this pattern was the PRE whose EMC though second highest at zero voltage (89.24%) decreased to 80.55 at 240 V (Table 1). During the preparation of the PRE, the seeds were crushed, soaked, subjected to extremes of pH with salts that possibly affected ionic positions. These predisposed the protein molecules to denaturation which from the account of workers like Gilles (2006) could expose more surfaces of the protein structure, enhancing their EMC. When these protein molecules were subjected to hydrolysis, as with sample C, the exposure could even be enhanced, increasing further the EMC (Table 1).

Stability (%) of emulsions prepared at varying voltages in current carrying and Ohmic heating beakers

The emulsification stability at 0 V or normal conditions was 22, 23, and 36%, for whole okra seed flour, PRE, and hydrolyzed PRE, respectively. It was a consistent improvement though marginal. That of gelatin at the same conditions was 45%. Micheal (1990) explained that there is a positive correlation between protein solubility and emulsion capacity/stability. Non dissolved protein contributes very little to emulsification. Protein must dissolve and migrate to the interface, before their surface properties could come into play. The denatured and

hydrolyzed protein molecules dissolve more readily and provide better coating for the dispersed phase, resulting in enhanced ES (Fennema, 2003).

At higher voltages, the samples generally showed increased ES. At 200 V, the percent emulsion stability of sample A (whole okra seed flour), was 25 at CB and 31 at OB. These are significantly different from that of PRE sample B (43% in Ohmic heating beaker). In current carrying beaker, sample B had ES of 25%. Sample C (hydrolyzed PRE) had a significantly higher score (98%) of ES as compared to the other okra seed products at 200 V. Under the same condition, gelatin also scored 98% ES.

For the emulsions prepared at 210, 220, and 230 V, the ES scores for PRE sample B (in current carrying beaker-CB) were, respectively 43, 46, and 50%. Under the same conditions, gelatin scored 98, 98, and 98%, respectively. In Ohmic heating beaker-OB, the scores are, respectively 43, 65, and 75%. For gelatin, the corresponding scores are 65, 75 and 80%. Ionic emulsifiers produce emulsions having a dispersed phase that exhibit particle charge. Proteins particularly the hydrophilic portions are ionic and can introduce particle charge and conductivity to emulsions. Under such conditions, solubility and therefore emulsion capacity and stability could be enhanced. These are likely to be the reasons for the increasing stability of emulsions produced in Ohmic heating and current carrying beakers (Balsam, 1994).

The ES for the hydrolyzed PRE was significantly higher than those of other samples in the experiments performed in a current carrying beaker (conductor). At 200 V for example, while the emulsions prepared with samples A and B had 23 and 25%, respectively that of sample C (hydrolyzed PRE) had 98%. This is quite remarkable judging from the fact that at 0 V, the same sample C made emulsion of only 36% stability. The interactions of the hydrophilic and hydrophobic groups

Table 2. Emulsion stability (%) of emulsions prepared at different voltage of electricity (in CB and OB).

Sample	Emulsion stability (%)					
	0	200	210	220	230	240
A _{CB}	22 ^a	23 ^a	24 ^a	26 ^a	26 ^a	25 ^a
A _{OB}	22 ^a	31 ^a	42 ^b	50 ^b	58 ^b	70 ^c
B _{CB}	23 ^a	25 ^a	43 ^b	46 ^b	50 ^b	52 ^b
B _{OB}	23 ^a	43 ^b	65 ^b	75 ^c	83 ^c	87 ^c
C _{CB}	36 ^a	98 ^c	98 ^c	98 ^c	98 ^c	98 ^c
C _{OB}	36 ^a	61 ^b	67 ^b	75 ^c	80 ^c	85 ^c
D _{CB}	45 ^b	98	98 ^c	98 ^c	98 ^c	98 ^c
D _{OB}	40 ^b	65 ^b	75 ^c	80 ^c	81 ^c	90 ^c

A: Whole okra flour; B: protein rich extract; C: hydrolyzed protein rich extract; D: gelatin; CB: current carrying beaker; OB: Ohmic heating beaker; Sample on same column with same subscripts are not significantly different ($p \leq 0.05$).

Table 3. Comparing okra seed PRE and gelatin as emulsifiers (o/w and w/o).

Dispersed phase Conc. (%)	Okra seed-PRE (EMC)		Gelatin (EMC)	
	w/o	o/w	w/o	o/w
5	6.0 ^a	84.0 ^a	1.90 ^a	94.9 ^a
10	7.3	81.32 ^a	10.90 ^c	86.4 ^a
15	13.3 ^a	77.0 ^a	19.0 ^b	83.9 ^a
20	16.0 ^b	73.0 ^a	24.2 ^b	80.5 ^a
30	20.0 ^b	72.7 ^b	10.0 ^c	79.4 ^b

w/o: Water in oil emulsion; o/w: oil in water emulsion; PRE: protein-rich extract; EMC: emulsification capacity. Figures on same column with same subscripts are not significantly different ($p \leq 0.05$)

are likely to be quite higher for sample C as compared to A and B. The stability was relatively higher for both samples B and C as compared to A. For emulsion production done at Ohmic heating beakers as compared to those prepared in current carrying beaker, the stability 62% is significantly lower than 98% which sample C had (Table 3). Figure 4 shows okra product- hydrolyzed okra seed PRE (sample C) prepared at 200 V, in a current carrying beaker-CB. It had an EMC of 94% and ES of 98%, a combination that was only attempted by gelatin at the same conditions. EMC and ES for sample C remained almost the same at 210, 220, 230, and 240 V (Tables 1 and 2 and Figure 4).

Okra seed PRE in O/W and W/O emulsions

The results of comparative analysis of PRE and gelatin as emulsifier at both 95% oil level (water in oil) and 95% water level (oil in water) situations are shown in Table 3. EMC for both okra seed extract and gelatin were higher

at lower oil levels, decreasing steadily with increasing concentration of oil. Balsam (1994) stipulated that this could be a pointer to the fact that okra PRE is a hydrophilic emulsifier. At lower levels of water content (5%), that is, 95% oil content, okra PRE performed better than gelatin. The same happened at 20% water. Maybe due to multiple structural nature of vegetable proteins (particularly after denaturation and other forms of alterations that took place during extraction), the PRE might have more than one structural forms, some of which might be more lipophilic as compared to gelatin.

The dispersed phase in the water in oil (W/O) emulsion is water. At 5% water level, the EMC for PRE is 6 and 1.9 for gelatin. At 10 and 20% oil levels, it is 7.3 and 16.0, and 10.9 and 24.2, respectively for PRE and gelatin. The oil in water version was significantly different. At 10 and 20% oil levels, the EMC (%) for PRE and gelatin were respectively 81.3, 73.0, 86.4, and 80.5. This is an indication to the fact that okra seed PRE just like gelatin are hydrophilic emulsifying agents (Micheal, 1990). This means that the hydrophilic lipophilic balance might be in

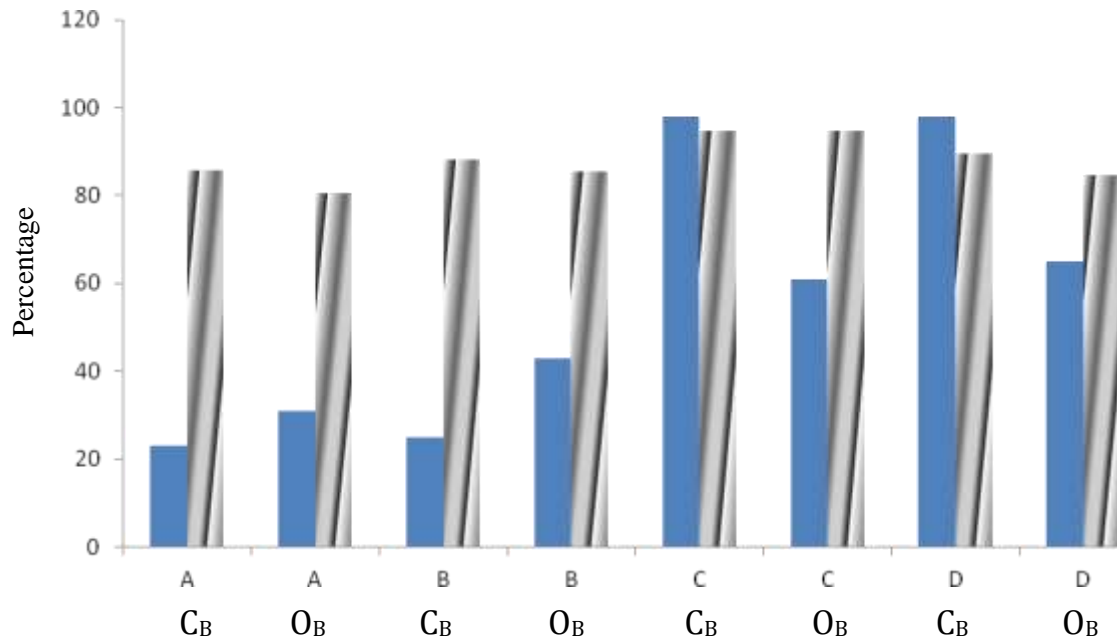


Figure 4. Emulsification capacity and Stability of emulsions (made at 200v). Striped column: emulsification capacity (%); Blue column: emulsion stability (%). CB: Current carrying beaker; OB: Ohmic heating beaker; A: whole okra seed flour; B: protein rich extract (PRE); C: hydrolyzed PRE.

the range of 11 to 12. They are more effective in the preparation of oil in water emulsions like kola cream soups stews, etc.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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