

# Effects pH and NaCl on the Protein Solubility, Emulsifying and Foaming Properties of Germinated and Ungerminated Melon (*Colocynthis citrullus*) Seed Flour

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**Abstract:** Defatted flour was prepared from germinated and ungerminated melon seeds. Protein solubility, foaming and emulsification capacities were measured over the pH range of 2.0 to 11.0 and NaCl concentrations of 0.1 M to 1.0 M. Below and above pH 4, protein solubility increased reaching maximum values of 60% and 70% for ungerminated and germinated flour respectively at pH 10.5. The emulsification capacity versus pH profile and the foaming capacity versus pH profile showed close resemblance to the protein solubility versus pH curve suggesting that those properties depended on solubilized protein. Protein solubility of ungerminated and germinated flour was reduced at higher salt concentrations of 0.6M and 0.8M respectively, which also altered the emulsifying and foaming properties. The germinated flour showed higher protein solubility, foaming and emulsification capacities than the ungerminated flour at all pH and NaCl levels, may be due to protein modification occurring during germination.

**Keywords:** Functional Properties, Mellon Seed, NaCl, pH, Flour

## 1. Introduction

Melon (*Colocynthis citrullus* L.) is cultivated extensively in the Eastern parts of Nigeria. Traditionally it is used as protein source and thickening ingredient in local diets. Studies showed that melon has potentials of been a household functional ingredient alongside soybeans in modern foods (Akobundu et al 1982). Protein, the principal constituent of legumes, determines largely the functionality of legume flour. Proteins are generally functioned in the presence of a liquid-water phase and therefore their interaction with water is the first and perhaps the most critical step in imparting their functionality. Functional properties of protein such as swelling, elation, solubility, emulsification and water holding capacity are found to be directly related to the manner by which protein interacts with water (Koury and Spinelli 1975; Chou and Morr, 1979). However, interaction of water with protein is affected by several factors such as the number and the nature of the building sites on the protein molecules, protein conformation, and the physicochemical environment

such as pH, salt and temperature (Chou and Morr, 1979).

While legume flours such soybean flour had been extensively studied, literature on the effects of those physicochemical environments on the functional properties of melon seed flour is awfully fragmentary. The present study was undertaking to evaluate the effects of pH and salt (NaCl) concentration on the protein solubility, emulsifying and foaming properties of germinated and ungerminated melon seed flour.

## 2. Materials and Methods

Two kilograms of dry melon seeds (*Colocynthis citrullus*) were purchased from local food dealers in Owerri, Nigeria. The seeds were sorted and extraneous matter removed. The bulk was divided into two portions; one portion for germination and the other for direct experimental use.

**Germination:** One kilogram of melon seeds was soaked in 20 litres of water for 16 hours (overnight). After soaking, the seeds were washed, spread thinly on moist wooden plane and

covered with moist cloth. Seeds were incubated at 30°C for 72hrs in the dark. During the incubation, seeds were sprinkled with water at intervals of 18hrs. At the end of the germination period, ungerminated seeds were removed and sprouts thoroughly washed and sundried.

**Flour Preparation:** The seeds were manually dehulled, and milled using a domestic grinder. A modified method of Anusuya-devi and Venkataraman (1984) was adopted for defatting the meal. The meal was defatted with food-grade n-hexane (three successive extractions by steeping). A solute: solvent (n-hexane) ratio of 1:5 was maintained. Fifty gram of the melon meal was weighed into a separating funnel. Then 250ml of n-hexane was added to the meal, and the mixture vigorously shaken for about 10minutes, with gases released at intervals. The homogenous mixture was allowed to sediment and the liquid phase decanted. This process was repeated. The clarity of the liquid phase after the second and third decanting indicated the extraction was complete. The defatted cake was poured into a clean white nylon cloth and the absorbed solvent squeezed out. The defatted material was spread in tray and dried in Gallenkamp moisture Extraction oven (model OVH 500) at a temperature of 50°C to a moisture content of 7 percent. Before analysis, flour was passed through an 80-mesh sieve using an Endecot siver shaker (model EVHI).

**Protein Solubility:** The protein solubility of the germinated and ungerminated melon flour was determined using a modified procedure of Narayana and Rao (1982). One gram of flour was mixed with distilled water; in a flour water ratio of 1:60. The mixture was stirred for 2hrs using magnetic stirrer (PHYWE MODEL). The pH of the suspensions were adjusted by the addition of 2N HCl or 2N NaOH. The suspension was centrifuged for 30minutes at 400rpm. Proteins of supernatant were determined by Micro-Kjeldahl method. Protein solubility was determined as a function of pH and NaCl concentration.

**Emulsification Capacity (EC):** Emulsification capacity was determined by the method of Beuchat *et al.* (1975) with slight modification. Two grams of flour samples were mixed with 10ml distilled water and stirred with a magnetic stirrer (PHYWE MODEL) at rührer speed for 30 seconds. After complete dispersion, refined vegetable oil (ANGEL BRAND) was continuously added from a burette and blending continued until the emulsion point was reached, when there was a separation into two layers. Effect of pH on the emulsification capacity was evaluated after adjusting the pH to the desired values with 2N HCl or 2N NaOH. Emulsification was also determined as function of NaCl concentration.

**Foam Capacity (FC):** The foam capacity of the flour samples was determined as described by Lawhon *et al.* (1972). Five grams of flour samples were mixed with 100ml distilled water in a beaker. The suspension was stirred for five minutes at 10 round speeds, with a magnetic stirrer (PHYWE MODEL). The suspensions together with the foam produced were poured into a 250ml measuring cylinder. The volume of foam after 30 sec was expressed as foam capacity.

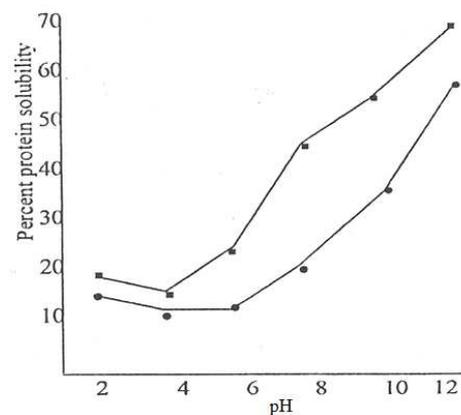
Percent volume increase was calculated according to the following equation.

$$\text{Percent foam volume} = \frac{\text{Volume after whipping} - \text{Volume before whipping}}{\text{Volume before whipping}} \times 100$$

The effect of pH and NaCl concentration on foaming properties was investigated. The pH of the suspension was adjusted to desired values by the addition of 2N HCl or 2N NaOH.

## 3. Result and Discussion

### 3.1. Protein Solubility



**Fig. 1.** Protein solubility-pH profile of germinated and ungerminated melon seed

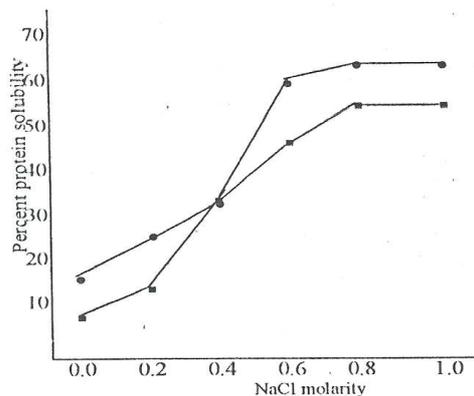
■ Germinated; ● ungerminated

Effects of pH on the protein solubility of germinated and ungerminated melon flour are shown in Fig1. Both the ungerminated and germinated flour had minimum solubility at pH4. On both side of the pH4, the solubility increased, reaching the maximum value of 60% and 70% at pH 10.8 for ungerminated and germinated flours respectively implying that most of the proteins were soluble in the alkaline region. Similar phenomena had been reported by Bena and Mukherge (1989) who observed that on the acid side of the isoelectric pH, there was only a small increase in solubility while on the alkaline side there was a steep increase in solubility in the case of Rice bran protein concentrate. This trend could be because pH affects charge and electrostatic balance within and between the protein, and solubility tends to be minimum of the isoelectric point where the net charge is zero (Schnepf, 1992). At this point attractive forces predominate and molecules tend to associate; away from this isoelectric point, the protein molecules are charged, and solubility and dispersability are enhanced.

At all the pH levels studied, the germinated flour exhibited greater solubility than the ungerminated flour. Since highly proteolysis activity within the germinating seed might be expected, an increase in the protein solubility resulting from the hydrolysis of the storage protein would be realistic. King

and Puwastein (1987) stated that even low amounts of proteolysis activity may be sufficient to break a number of critical popicles bonds and thereby produce a notable change in solubility distribution. Although peptide bond cleavage may have played a central role in the solubility, Lukow and Bushuk (1984) noted that degradation of carbohydrate and/or lipids associated with the specific proteins could disrupt the native protein structure and generate smaller more soluble aggregates.

The effects of NaCl concentration on the protein solubility of the germinated and ungerminated flours are presented in Fig 2. Solubility of both flours increased with higher concentrations of NaCl levels up to 0.6m and 0.8m for ungerminated and germinated samples. Beyond these NaCl concentration levels, there was observed increase in the solubility of the flour samples. Neutral salt has been known to have a two-fold effect on protein solubility. At low levels, it increases solubility by suppressing the electrostatic protein – protein interaction (salting –in effect). At higher concentration, the protein solubility is decreased due to the ionic tendency of the salt which creates competition between the protein and added salt (salting-out) (Nevin, 1978). The variations in the two curves may be due to differences in the characteristics of the protein in salt solutions (Narayana and Rao, 1982).



**Fig. 2.** Effects of NaCl concentration on the protein solubility of germinated and ungerminated melon seed flour

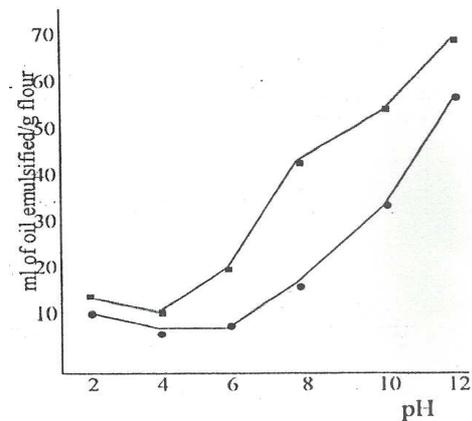
▪ Germinated; • ungerminated

### 3.2. Emulsification Capacity (EC)

The emulsification capacity of germinated and ungerminated melon as a function of pH is shown in Fig3. The pH- emulsification capacity curve of both flours closely resembled the protein solubility versus pH profile, suggesting that emulsification capacity depended on the solubilized proteins. The minimum EC values were 11ml/g and 6ml/g flour for germinated and ungerminated flours respectively at pH 4.0. Both flours showed the highest EC values at the alkaline region. Dependence of emulsification capacity on pH is expected as it is known that emulsification of soluble proteins depends on hydrophilic and lipophilic proteins (Sasulski et al., 1976). Similar relations are reported for

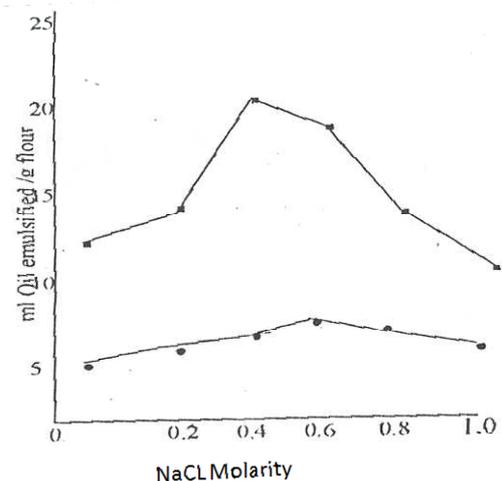
soybean proteins (Crenwelge et al., 1974), groundnut protein (Ramanathan et al., 1978) and guar protein (Nath and Rao, 1981). The EC of the germinated flour is much better at all levels of pH. This can be attributed to the fact that germination modifies seed proteins and increases their solubility, and emulsification is a reflection of the solubilized protein (King and Puwastein, 1987).

The melon flours formed basically two types of emulsions. At the lower pH region (pH 2-6), thin emulsions were formed but as the pH level approached 10.5 – 11.5, very thick emulsions were formed. Cherry et al (1978) stated that the viscosity of emulsions was related to the types of soluble proteins in the suspension.



**Fig. 3.** Effects of pH on Emulsification capacity of germinated and ungerminated melon seed flour

▪ Germinated; • ungerminated



**Fig. 4.** Effects of NaCl concentration on emulsification capacity of germinated and ungerminated melon seed flour

▪ Germinated; • ungerminated

The influence of Nacl concentrations on the EC of the germinated and ungerminated flour is reported in Fig 4. The EC values of both flours increased with more incorporation of Nacl, up to 0.4M and 0.6M NaCl concentrations for the ungerminated and germinated samples respectively. Beyond

these concentrations levels, the EC values dropped. A similar effect of salt concentration on alfalfa leaf proteins was reported by Wang and Kinsella (1976). This is probably due to the salting-in effect of NaCl on proteins. At higher concentrations there is also drop in EC values as there is likely to be salting out effect on the proteins. Shanmugasundaram and Venkataraman (1989) also reported a similar slight decrease in the emulsification capacity of detoxified madhuca flours after 0.2M NaCl concentration.

### 3.3. Foaming Capacity (FC)

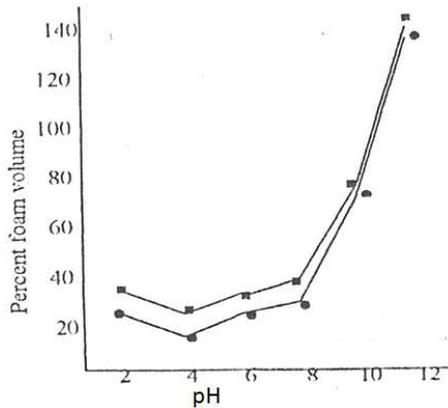


Fig. 5. Effects of pH on Foam capacity (FC) of germinated and ungerminated melon seed flour

■ Germinated; ● ungerminated

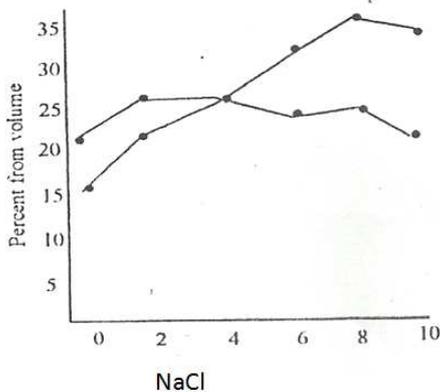


Fig. 6. Effects of NaCl concentration on the Foam Capacity of germinated and ungerminated melon seed flour

■ Germinated; ● ungerminated

The foaming capacity versus pH profile of the germinated and ungerminated flour as shown in Fig 5 had a close pattern to the protein solubility-pH curve, suggesting that foaming property is also dependent on the solubilized protein. Minimum FC values of 20% and 12% were recorded for germinated and ungerminated flours respectively at pH 4. The foaming capacity of the germinated and ungerminated samples increased with increase in pH reaching maximum values of 120% and 128% respectively at pH 10. Devi and Venkataraman (1984) repeated minimal FC values at pH 3 and pH 4.5 for Blue-green Algae and soybean respectively,

while noting maximum FC values at pH 10 for both samples.

Similar observations have also been reported in the case of soy protein isolate, caseinate, and whey protein concentrates (Hermansson, 1975). The FC values in both the alkaline range and acid range were greater for germinated flour than for and ungerminated flour, which may be done to more solubilized protein caused by modification during germination. Formability if related to the rate of decrease of surface tension of the air/water interface caused by adsorption of protein molecules.

The addition of NaCl improved the foaming capacity of the germinated and ungerminated samples (Fig 6). Maximum foaming was observed at salt concentrations of 0.4M and 0.8M for ungerminated and germinated flours respectively. Sodium chloride had been reported to enhance the foaming of soy protein (Eldridge *et al.*, 1963). Such increase in foaming capacity due to the addition was attributed to the increased protein solubility. According to Bera and Mukherjee (1989), salt, depending upon their concentration probably affect foaming by enhancing solubility initially, whereas salting-out may occur when high concentrations are used.

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