Solubility of Rapeseed Protein in Aqueous Solutions

M.N. RADWAN and **B.C.-Y. LU**, Department of Chemical Engineering,
University of Ottawa, Ottawa, Ontario, Canada

ABSTRACT

The solubility of the protein of the dehulled and defatted 'Tower' variety of rapeseed in aqueous solutions was determined at temperatures of 25, 35, 45, and 55 C in the pH range of 1-13. It was found that the points of minimum solubility occur at pH values of 4.5, 4.8, 7.0, and 7.2, respectively, for the above four temperatures. No color change of the meal was observed at or near the minimum solubility point. The aqueous solutions were prepared at the selected pH values by using either NaOH or H_2SO_4.

INTRODUCTION

Canada is one of the major world producers of rapeseed, which is used primarily for its oil (1-3). The meal remaining after oil extraction contains protein (ca. 50%), carbohydrates, fibers (mainly from the hulls), thioglucosides, and a small amount of residual oil. Because of a general worldwide shortage of protein and an increasing demand, rapeseed offers a very appealing solution in its high protein content.

The technology of rapeseed oil extraction presently in use does not include a suitable procedure to leave the protein in the meal in a digestible form, free from any deleterious matter. The presence of the indigestible fibrous material and the thioglucosides is the main impediment to the use of the meal as food for human and animal consumption (4-7). Other workers have successfully reduced the fiber contents in the product by crushing the seeds and separating the hulls by air classification (4). The thioglucosides are hydrolyzed by enzymic action to the toxic isothiocyanates, nitriles, and thiocyanates (8).

Tests on a variety of animal species (9), including human beings (10), have shown that these compounds are goitrogenic. They also affect the adrenal cortex, the pituitary, the kidney, the liver, and cause the thyroid adenoma with the effect on ruminant animals being less marked than that on nonruminants (11). It has been observed that they cause a decrease in the rate of growth of the animals and, in some cases, in the amount of milk produced. The milk, which is contaminated with the toxic thioglucosides (9,11), cannot be transformed into its other useful products, since the toxic compounds may poison the milk bacteria (12).

The detoxification of the meal is an important step, and several attempts have been suggested in the literature to achieve this end. One method used is that of plant breeding. In Canada, a new variety called 'tower' *(Brassica napus)* which contains only ca. 0.15% thioglucosides has been produced by this means. Although this low concentration of deleterious matter is acceptable for animal feed materials (13; T.T. Hartsock, private communication), it is not suitable for human consumption. In commercial practice, the enzyme is deactivated by heat in order to prevent the appearance of the toxic compounds (6).

Nevertheless, the residual enzyme activity may hydrolyze the thioglucosides; the same result can occur as a result of the introduction of the enzyme by ingestion with the food or its synthesis by bacteria in the intestinal tract of the host species (6,11).

An alternative method of purification is based on deactivation of the enzyme in boiling water for a few minutes followed by aqueous extraction of the toxic compounds (6). A good quality protein was obtained, free from the toxic compounds, but with a reduced protein yield.

The purpose of this work is to determine the conditions of minimum solubility of the rapeseed protein in aqueous solutions in order to remove the toxic compounds and to minimize the protein loss.

Throughout the text, the words "meal" and "meat" refer to "defatted" and "dehulled" seeds, respectively.

TABLE I

Composition of 'Tower' Rapeseed Meal[a]

	As received (%)	Dry basis (%)
Water content	7.75	---
Fat	0.55	0.60
Nitrogen	7.61	8.25
Protein (N_2 x 6.25)	47.55	51.54
Fiber	4.34	4.71
Ash	9.39	10.18
Carbohydrate (by difference)	22.81	24.72

[a]The analysis was done in the Food Research Institute, Department of Agriculture, Ottawa, Canada

MATERIALS AND METHODS

The new variety of rapeseed 'Tower' was used in this investigation. The seeds were cracked but not ground. The hulls and the meat fines (≅ 30% of the seed mass) were removed by air classification. The oil (≅ 42% of the meat) was extracted by hexane for 23-30 hr in a Soxhlet extractor. The meal was air dried and any remaining solvent was completely removed under vacuum (< 1mm of Hg) at room temperature for 1 hr. The composition of the meal is presented in Table I.

The protein solubility experiments were carried out in a constant temperature shaker water bath (Blue M "Magni-Whirl") capable of controlling the water temperature to ± 0.2 C, over the range of 5-100 C.

The pH and electrode potentials were measured using an Accumet 420 digital pH/Ion meter equipped with a temperature compensator. This instrument is capable of measuring the pH to the second decimal place, and on the expanded scale for millivolts reads to the first decimal.

An Orion Ammonia Electrode model 95-10 which is capable of detecting dissolved ammonia in aqueous solutions over the concentration range of $1\text{-}10^{-4}$M was used to measure the ammonia content of the digested supernatant. The theory of operation of the ammonia electrode is available in the literature (14-17).

The experiments were done in aqueous solutions of either sulfuric acid or sodium hydroxide over a pH range from 1 to 13 and at temperatures of 25, 35, 45, and 55 C.

Meal (1 g) and the prepared solution (50 ml) were placed in a stoppered 125 ml conical flask and shaken 1 hr in the constant temperature shaker bath. The agitation time of 1 hr was chosen because it was found in two tests that a constant pH value was reached in < 30 min. Thus, the 1 hr period ensures that a constant pH is reached for all samples. The 1 hr shaking period was adopted by some authors (18), while in other work (19,20) only 30 min was used.

After shaking for 1 hr, the supernatant was decanted and centrifuged for 5-6 min at 2,500 rpm. To a 10 ml sample of the supernatant, 0.7g HgO, 15g powdered K_2SO_4, and 25 ml concentrated H_2SO_4 were added together in a Kjeldahl flask. The sample was digested until the solution became clear and for an additional 30 min (21). After cooling, the contents were transferred quantitatively to a

1,000 ml volumetric flask, and distilled water was added to volume. An aliquot of 20 ml of the dilute digested sample was transferred to a 150 ml beaker, then 2 ml of an alkaline reagent (10 M NaOH + 2 M NaI) and ca. 80 ml of distilled water were added. The NaI was added to complex the mercury catalyst and free the ammonia. Care was taken to maintain the temperature of the solution at 25 C during the addition of the alkaline reagent and the distilled water. The solution was stirred at a low speed to avoid the formation of a vortex. The ammonia electrode was placed in solution immediately after the stirring was started. One minute after placing the electrode in the solution, a reading on the expanded scale (± 199.9 mV) was teken (E_1). Then 10 ml of a standard solution (NH_4 Cl, 20 ppm as nitrogen) was added. After another minute, a new reading was recorded (E_2). The difference in the potentials ($\Delta E = E_2 - E_1$) corresponds to a value, Q, which is the concentration ratio of unknown to known in Table II of the Instruction Manual supplied by the manufacture (14). When this tabular value of Q is multiplied by 500, the concentration of nitrogen, in mg, in the solution was obtained. The analysis was repeated several times, and the results were found to be reproducible within ± 0.2 mV.

At the beginning and end of each series of measurements, the electrode was checked using a digested solution of glycine; the error was found to be $< \pm 2\%$. A blank was prepared by digesting a small amount of solid dextrose (ca. 1 g). The nitrogen content of the blank was too small to be measurable, showing that there would be no need to purify the distilled water used. The analysis was completed by measuring the pH of the remaining supernatant.

RESULTS AND DISCUSSION

The experimental results and the conditions under which they were obtained are shown in Figure 1. Table II summarizes the temperatures and the pH values where the minimum solubilities were obtained. The minimum solubility increases with temperature, and the pH values corresponding to these minima also increase with temperature. This is due to changes in the dissociation constant of the protein with temperature. A higher temperature allows the hydrophobic amino acids in the interior of the protein to become exposed to the solution. In this new configuration they are thus able to affect changes in the shape of the solubility curve.

It is noted that the meal exert some buffering action, particularly at pH values around the minimum solubility. It was observed in this investigation that no color change of the meal occurred at or near the minimum solubility. In other regions, the color of the meal changed gradually from yellow to gray in the direction of increasing acidity, and from yellow to dark green in the direction of increasing alkalinity. Similar color behavior was observed, only more pronounced, in the extract.

In Figure 1, it can be seen that, in general, the protein solubility increases by going to the extremes of pH. At a temperature of 35 C and in the vicinity of pH of 1, a decline in the solubility occurs; the same phenomenon is observed at a pH near 13 and at temperatures of 45 and 55 C. These effects are attributed to the denaturation of the protein.

At temperatures of 45 and 55 C and at pH values < 2, nitrogen solubility increases. At 45 C, this increase occurred after passing a shoulder. The increase of the solubility at these conditions can be ascribed to either partial hydrolysis of the protein or fracture of the seed cell wall with the release of new sources of nitrogen or both.

In the work of Korolczuk and Rutkowski using rapeseed (20), the effect of two protein groups is more pronounced and may be due to the method of preparation of the seeds

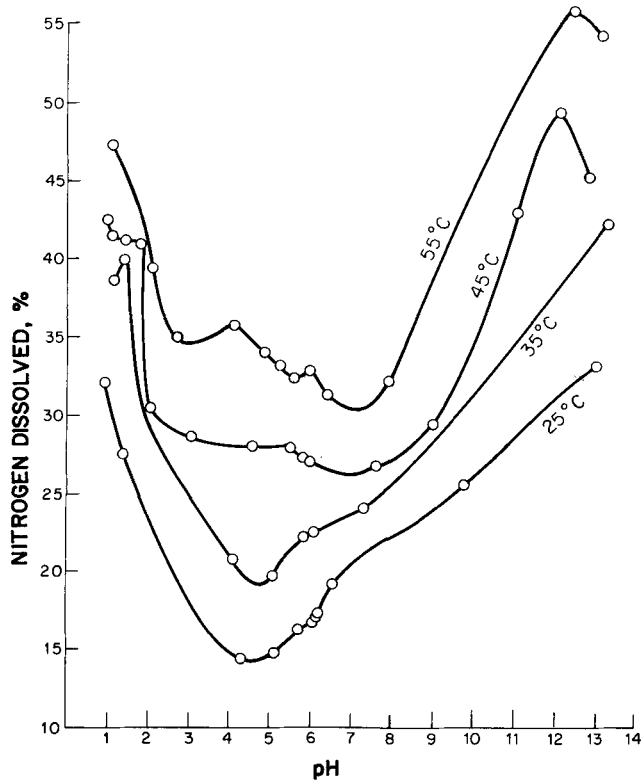

FIG. 1. Solubility of rapeseed protein in aqueous solutions, expressed in terms of the amount of nitrogen dissolved, as a function of pH and temperature.

TABLE II

Minimum Solubility of Protein of 'Tower' Rapeseed in Aqueous Solutions

Temperature (C)	pH	Minimum solubility (%)
25	4.5	14.2
35	4.8	19.0
45	7.0	26.2
55	7.2	30.2

or to differences in the variety of seed used. By rearranging their Figure 1 to be the percentage of nitrogen extracted versus pH at constant temperature, the appearance of two protein patterns and their effect on the solubility are observable.

The results of this investigation can be related to similar work in the literature. It has been found that, at room temperatures, minimum solubility using soybean occurs between pH 3.5 and 4.5 (18,19,22). Using radish meal (22) and Crambe abyssinica (18), both of which are in the family Cruciferae with rapeseed, the corresponding minima are found to be between pH 3.8 and 4.3 and between pH 4 and 4.2, respectively, at the same temperature. A reported value in the work of Sosulski and Bakal (23) for rapeseed, flax, and sunflower is in the pH range of 4.4-4.6. From Table II it is seen that under similar conditions 'Tower' rapeseed yields a value of pH 4.5, which is in good agreement with the values reported in the literature.

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