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Extractability of polyphenols of sunflower seed in various solvents

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Abstract. The extractability of chlorogenic acid from defatted sunflower seed flour in water and salt solutions at different pH values and also in aqueous organic solvents was determined. It increased with increase in pH and at pH 8 in water nearly 70% chlorogenic acid was removed in a single extraction, while NaCl did not increase the extraction, and, $MgCl_2$ and $CaCl_2$ increased it, especially at higher concentrations. Methanol, ethanol, isopropanol and acetone, at 20% concentration in water, caused the maximum extraction of polyphenol. These organic solvents without added water were poor solvents for the extraction of polyphenol from the flour.

Keywords. Polyphenol; sunflower seed; extractability of chlorogenic acid.

Introduction

The presence of chlorogenic acid as a major polyphenol in sunflower seeds has been well recognised (Smith and Johnsen, 1948; Joubert, 1955; Sechet *et al*., 1959; Milic *et al.*, 1968). Caffeic acid and quinic acid are also present in sunflower seeds (Sechet *et al*., 1959; Milic *et al*., 1968). These cause discolouration of sunflower proteins at alkaline pH (Smith and Johnsen, 1948) and also lower the nutritive value (Horigome and Kandatsu 1968; Jung and Fahey, 1981) because of their interaction with residues such as lysine, cysteine, methionine (Pierpoint, 1970), alanine, phenylalanine and glutamic acid (Nakatani and Kurasawa, 1979). Many methods have been tried to remove the polyphenols from sunflower seed using aqueous, organic and aqueous— organic solvents (Smith and Johnsen, 1948; Joubert, 1955; Mikolajczak *et al*., 1970; Pomenta and Burns, 1971; Sosulski *et al*., 1972, 1973; Fan *et al*., 1976; Sodini and Canella, 1977). Reducing agents have also been used to prevent oxidation of chlorogenic acid during isolation of the proteins (Gheyasuddin *et al*., 1970). In these methods complete removal of chlorogenic acid is not achieved in a single extraction and repeated extraction is necessary to achieve even partial removal. The hydrogen bond between hydroxyl groups of phenolic compounds and peptide bonds in protein is known to be unusually strong and equilibrium in aqueous solutions strongly favours the formation of complexes between phenols and proteins (Loomis and Battaile, 1966).

Inspite of a large number of methods for removal of polyphenols (especially chlorogenic acid) from sunflower, no systematic attempt appears to have been made to study the extractability of the polyphenols in various aqueous and

aqeuous-organic solvents as a function of solvent composition, pH, temperature, etc. The present investigation attempts such a systematic study. The choice of the solvent system has been guided by the solubility of the polyphenols in various solvents as indicated in the literature.

Materials and methods

Materials

Sunflower: Sunflower seeds, the Russian variety EC 68415, grown in the State of Karnataka, were obtained from Karnataka State Agro Seed Corporation, Mysore.

Chemicals: Chlorogenic acid, caffeic acid and quinic acid used as standards were from Sigma Chemical Co., St. Louis, Missouri, USA; NaCl, $CaCl_2$, $MgCl_2$, $NaNO_2$ and CH_3COOH were of GR grade from E. Merck, Dermstadt, Germany. Isopropanol, acetone, methanol and ethanol were from British Drug House Ltd., and were distilled once before use.

Methods

Sunflower seeds were dehulled in a centrifugal disc huller (Sastry, 1978) after drying at 50°C for 2 h. The kernels were flaked and defatted with *n*-hexane and the residual solvent was removed by air drying for 4-6 h at 30°C.

Estimation of protein

The nitrogen content was determined by microKjeldahl method (AOAC, 1975) and converted to crude protein by using a factor of 6.25.

Estimation of chlorogenic acid, caffeic acid and quinic acid: Defatted flake was ground in a Waring Blendor to pass 40 mesh sieve. Two *g* of the sample was used for the estimation of the acids according to the procedure of Pomenta and Burns (1971).

Since it was observed that chlorogenic acid was the major polyphenol (about 66%) in sunflower seed, to determine the efficiency of polyphenol removal from sunflower meal by various treatments only chlorogenic acid was estimated.

Removal of polyphenols

The following general procedure was used. The defatted sunflower meal (5 g) of 10 mesh sieve size were mixed with solvent in the ratio of 1 of flour to 10 of solvent (w/v) and stirred for 2 h at room temperature (about 28°C). The slurry was filtered and the residue on the filter paper was dried at 55-60°C in an oven till constant weight was obtained (5-6 h). The chlorogenic acid content of the residue was determined by the method of Pomenta and Burns (1971).

The effect of extraction with aqueous methanol, ethanol, isopropanol and acetone whose composition varied from 0-80%; NaCl, $CaCl_2$ and $MgCl_2$ solutions of varying molarity, water at different pH's and temperatures on removal of polyphenol was determined. The pH of water was adjusted with IM HCl or IM

NaOH. For determining the effect of temperature on the extractability of chlorogenic acid by water a shaking water bath whose temperature was controlled to ±1°C of the desired temperature was used.

When extractions were done in salt solutions, the residue after drying would contain the corresponding salt. Since the salts used were chlorides of different cations, the chloride content of the residue was estimated (Vogel, 1961), and the weight of the residue was corrected for the salt content.

Results and discussion

The chlorogenic, caffeic and quinic acid contents of sunflower meal given in table 1

Table 1. Composition of phenolic acids of defatted sunflower flour*.

Composition	*g*/100 *g* of flour
Protein	54.0
Chlorogenic acid	1.86
Caffeic acid	0.56
Quinic acid	0.39

* moisture free basis

indicate that chlorogenic acid forms the major polyphenol in the meal. The chlorogenic acid content of the meal was in the range of values of 1.42 to 4.00% reported for a number of sunflower varieties (Dorrell, 1976).

Only about 30% of chlorogenic acid was extracted in the pH range 2-3 (figure 1).

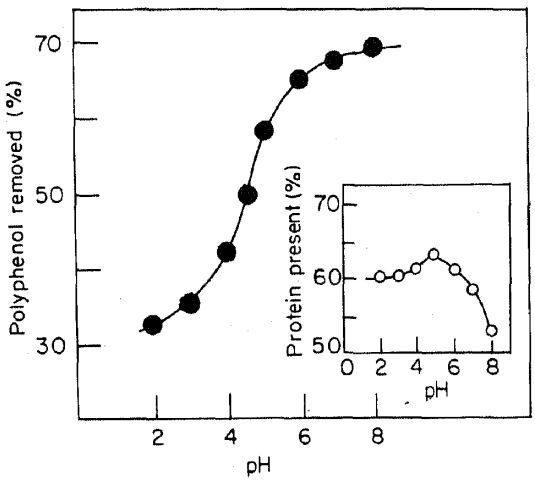

Figure 1. Effect of pH on the extractability of polyphenol in water. Inset figure gives the protein content of the flour.

However, above pH 4 there was a steep increase in the extractability of chlorogenic acid and reached a value of about 70% around pH 8. Polyphenols are known to undergo oxidation to the corresponding quinones at alkaline pH (Sondheimer, 1964). It is possible that the higher percentage of polyphenols extracted at alkaline pH could be due to the oxidation of the polyphenol and their inability to be estimated by the method of Pomenta and Burns (1971). To clarify this point the following experiments were done. The pH of a solution of chlorogenic acid in water was adjusted to different values upto pH 10 and set aside for 30 min at room temperature and the chlorogenic acid content was estimated (Pomenta and Burns, 1971). It was observed that upto pH 8 there was no change in the absorbance of solutions. However, above pH 8 the significant change in absorbance indicated that chlorogenic acid could not be estimated by the method of Pomenta and Burns (1971). This experiment clearly demonstrated that the data on the extractability of chlorogenic acid upto pH 8 were reliable and the data in figure 1 is not vitiated by any artifacts of assay. Thus it can be concluded that at pH 7 nearly 70% of chlorogenic acid could be removed by a single extraction.

The protein content of the meal which had been subjected to polyphenol extraction was also estimated. Upto pH 7 there was not much loss in the protein content of the meal (figure 1, inset). However, above this pH value there was considerable extraction of the protein also. These results indicate that by extraction at pH 6 to 7 most of the chlorogenic acid can be removed without loss in protein content.

The effect of NaCl, $CaCl_2$ and $MgCl_2$ was also tried on the a extractability of chlorogenic acid. The extraction with 0.1 M, 0.5 M and 1 M NaCl at different pH values did not result in increased removal of chlorogenic acid, but the amount extracted was lower. On the other hand, the addition of salt lead to greater losses in the protein content (figure 2, inset). This clearly suggested that extraction with

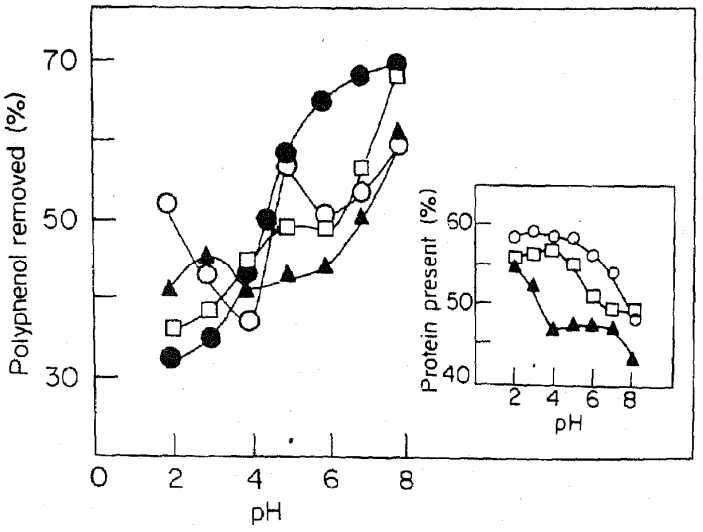

Figure 2. Effect of pH on the extractability of polyphenol at various concentrations of NaCl: (O) 0.1 M NaCl; (□) 0.5M NaCl; and (▲) 1 M NaCl. Inset figure gives the protein content of the flour.(●) indicates the effect of pH on the extractability of polyphenol in water alone.

salt solutions at different pH offers no advantages over the use of water. The result obtained with 0.05 M and 0.4 M $MgCl_2$ salts is given in figure 3. While at

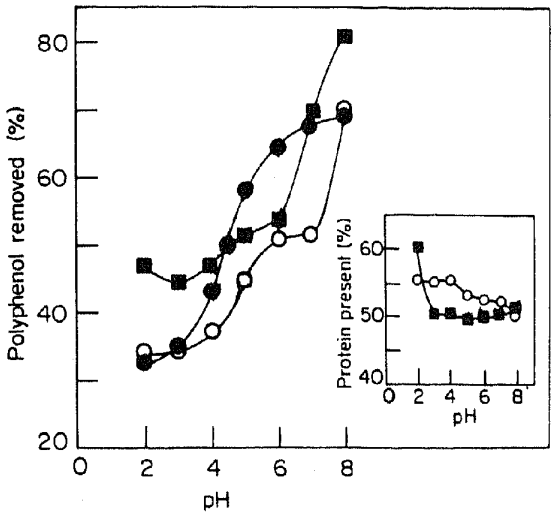

Figure 3. Effect of pH on the extractability of polyphenol at various concentrations of $MgCl_2$: (O) 0 05 M $MgCl_2$ and (■) 0.4 M $MgCl_2$. Inset figure gives the protein content of the flour.(●) Indicates the effect of pH on the extractability of polyphenol in water alone.

low $MgCl_2$ concentration there was no marked increase in chlorogenic acid extraction at a higher $MgCl_2$ concentration the extractability of polyphenols increased. In fact, at pH 8 nearly 82% of the polyphenol could be removed at 0.4 M $MgCl_2$. However, the extractability of the protein also increased and the protein content of the meal was much lower than that of the untreated meal. Thus while there was an advantage in the removal of the polyphenol there was a disadvantage so far as the protein content of the meal was concerned. Similar results were obtained with $CaCl_2$ also (figure 4). Here, in fact the extractability of the protein

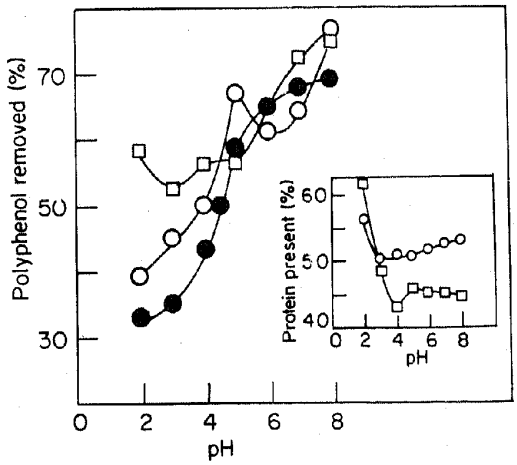

Figure4. Effect of pH on the extractability of polyphenol at various concentrations of $CaCl_2$: (O) 0.1 M $CaCl_2$ and (□) 0.5 M $CaCl_2$. Inset figure gives the protein content of the flour (●) Indicates the effect of pH on the extractability of polyphenol in water alone.

from the meal was much higher and there was considerable loss in the protein content.

The greater efficiency of salts, especially $MgCl_2$ and $CaCl_2$ to remove chlorogenic acid from sunflower flour may be due to their ability to interfere with any ionic linkages that may be formed between this acid and the protein. This inference is supported by the observation that increase in the salt concentration increases the efficiency of extraction and also the divalent salts have a greater efficiency than the monovalent salts like NaCl.

In figure 5 the effect of temperature on the extraction of chlorogenic acid is given. In the temperature range 30-50°C there was an increase in the extractability

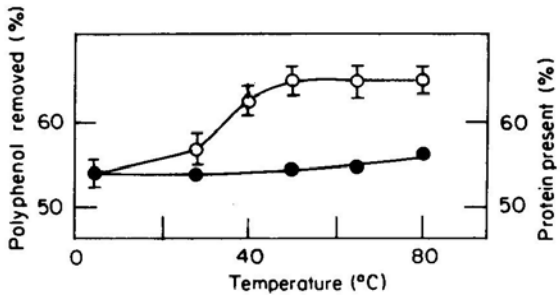

Figure 5. Effect of temperature (°C) on the extractability of polyphenol (○) Protein content of the flour after treatment with each temperature is also shown (●).

of chlorogenic acid; it increased from about 55% to about 65%. However, between 50-80°C there was no improvement in the extractability. The protein content of the meal also was more or less constant upto 65°C. At 80°C the protein content was slightly higher (56%). The increased extractability of chlorogenic acid as the temperatures increased is in accordance with the observation of Sosulski *et al,* (1972) who carried out the extraction only at 60°C and 80°C. However, this contradicts our observation that between 50-80°C there was no increase in extractability. This difference could possibly be due to differences in the varieties of sunflower, and also due to the difference in the technique used for extracting the polyphenols.

In figure 6 the extractability of chlorogenic acid from sunflower flour in aqueous-organic solvents is given as a function of the percentage of the organic solvent in the aqueous-organic solution. In methanol, ethanol, isopropanol and acetone the extractability increased upto about 20% and remained constant upto about 80%. However, above this concentration the extractability of chlorogenic acid decreases and in absolute ethanol and isopropanol the extractability was very poor being of the order of 10-12%. In pure methanol and acetone the extractability was slightly better (30%). These results clearly suggested that use of higher concentrations of alcohol did not offer any advantage in extracting chlorogenic acid. Joubert (1955) used 50% ethanol to extract the polyphenols. Smith and Johnsen (1948) used 70% ethanol or near absolute methanol to remove the acid. Similarly Fan *et al.*, (1976)

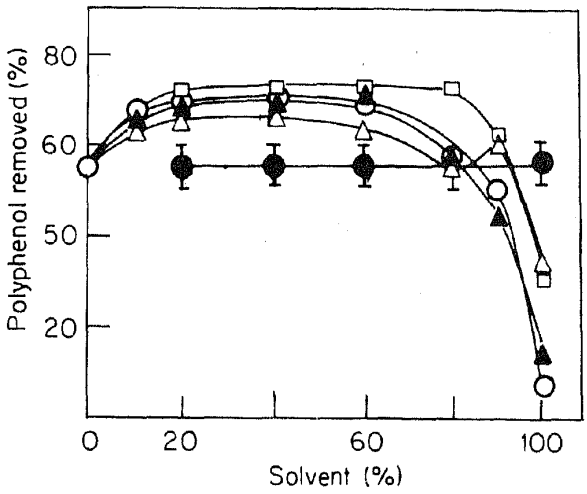

Figure 6. Effect of various organic solvents and various percentages aqueous-organic solvent mixtures in the extractability of polyphenols;(○) ethanol;(□) acetone;(▲) isopropanol; (Δ) methanol and (⧳) residual proptein in the flour after treatment with the above solvents respectively.

used 70% ethanol at 24°C. These authors used repeated extractions to achieve complete removal of polyphenol. These authors do not state why this composition of the solvents was used for extracting the polyphenols. Our results suggest that use of lower concentration of alcohol has an advantage over the use of higher concentrations. The use of lower concentrations of alcohol has the added advantage that the proteins are not likely to be denatured and possibly their physico-chemical and functional properties are not impaired. Figure 6 also shows that the protein content of the flour was not decreased by extraction at low concentrations of alcohol.

The low solubility of the polyphenols in absolute organic solvents may be due to strengthening of the hydrogen bonds between polyphenols and protein in these solvents (Sabir *et al.,* 1974). On the other hand the increase in solubility upon the addition of water to organic solvents could be due to the weakening of the hydrogen bonds in aqueous solutions. It could also be due to the increase in basicity (Bates *et al*., 1966; Paabo *et al.*, 1966; Brandes and Stern, 1968; 1976) and increased ionisation of the polyphenols in such solutions.

As mentioned earlier a number of methods have been attempted for removal of the polyphenol from sunflower meals. Sosulski *et al*., (1972) utilised 0.001N HCl for extraction. The pH of this solution would be approximately 3-4. It is not clear why acid condition was employed for extraction of chlorogenic acid. Our results indicate that extractability of the acid is much better at higher pH values and neutral pH. This is also confirmed by the observation that the binding of chlorogenic acid by sunflower 11S protein is. higher at lower pH than at higher pH (Sastry, Personal communication).

This investigation indicates that water or salt solutions at neutral pH or 20% organic solvents in water are efficient solvents for the removal of chlorogenic acid (and possibly caffeic acid and quinic acid) from sunflower meal.

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