

Proximate Composition, Nutritionally Valuable Minerals and the Effects of Some Salts on the Functional Properties of Silkworm (*Anaphe infracta*) Larvae

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Abstract. The investigations of the silkworm (*Anaphe infracta*) larvae on dry weight basis showed that total ash, crude fat and fibre values were low while crude protein and carbohydrate values were high. Fe, Zn, Mg and P were high while Na, Cu, Ni, K, Ca, Mn, Co, Cr were low. The lowest gelation concentration varied between 6.0 in 1.0% Na₂SO₃ and 14.0 in 20.0% NaCl, NaNO₃ and Na₂CO₃ with low CV%. All the water absorption capacity values were generally high, the highest being in NaNO₃. The oil emulsion capacities were generally low whereas the oil emulsion stability was good in all the salts. The isoelectric point under pH solubility depended on the type of salt solution under consideration. These results make *A. infracta* larvae useful in some food formulations.

Keywords: *Anaphe infracta*, chemical composition, salt effects, functional properties, nutritional value

Introduction

A number of insects or their products are to a certain extent still eaten by some West African tribes, as tit-bits, or exclusively by children. Such insects are mostly those which can be collected in large numbers, e.g. locusts in the gregarious phase, emerging termites, caterpillars and the large African cricket *Brachytrypes*. Also eaten occasionally and sometimes regarded as delicacies, are fatty 'grubs' such as the enormously distended queen termite and the larvae and pupae of scarabaeid beetles, and of the silkworm *Anaphe* spp. (Ene, 1963).

Such consumption, besides Africa, has been practised throughout the course of history and in all past cultures, including those of ancient China, Mexico, Egypt, Israel and Greece (Bodenheimer, 1951).

West African silk is made from the communal cocoons spun by the gregarious caterpillars of the moths *Anaphe infracta* (brown silk) and *A. imbrasia* (white silk). These caterpillars feed mainly on the leaves of *Bridelia micrantha* and *Albyzzia zygia*, respectively. The cocoon of *A. venata* is not used in making silk (African Encyclopedia, 1974; Ene, 1963). The first two species of caterpillars are popular as food among the Yoruba (Nigeria), and are sometimes offered for sale in the open markets. Since the *Anaphe* spp. are normally consumed in their seasons in Nigeria the intention of this study is to report the proximate composition, nutritionally valuable minerals and the effects of five different salts on the functional properties of silkworm (*Anaphe infracta*) larvae, for the benefit of the consumers.

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Materials and Methods

The caterpillars of *Anaphe infracta* were collected in the month of September around many trees of *Bridelia micrantha* in a farm located in Odo Ayedun-Ekiti, Ekiti State, Nigeria.

A traditional method was used for the processing of silkworm caterpillars. After collection (about 1 kg) they were roasted in a hot pot to remove the hair. They were then sun-dried for some days, screened and dry-milled to flour by using a Kenwood blending machine. The sample was preserved in a refrigerator until use for various analyses.

Sample analysis. The proximate analysis of the sample for moisture, crude fibre and total ash were carried out using the methods described by AOAC (1990). Nitrogen was determined by the micro-Kjeldahl method (Pearson, 1976) and the amount of crude protein was calculated (nitrogen content x 6.25). Carbohydrate was determined by the difference. Duplicate analysis were carried out.

The minerals were analysed from solutions obtained by first dry-ashing the sample at 550 °C to constant weight. Sodium and potassium were determined using flame photometer (Model 405, Corning, UK) and phosphorus was determined colorimetrically using a Spectronic 20 (Gallenkamp, UK) as described by Pearson (1976). All other metals were determined by means of an atomic absorption spectrophotometer (Pye Unicam Sp 9, Cambridge, UK).

Determination of functional properties. The variations of protein solubility against pH and salt concentrations were determined by the methods described by Oshodi and Ekperigin

(1989) as modified by Adeyeye *et al.* (1994) and a supernatant was obtained whose protein content was determined by the Biuret method (Wiechselboven, 1946). The salts used were sodium chloride, sodium sulphite, sodium nitrite, sodium carbonate and sodium ethanoate with their concentrations ranging between 0.5% and 20% w/v.

The Sosulski (1962) test procedure was followed to determine oil absorption capacity and the procedure of Sathe and Salunkhe (1981) was followed to determine the oil emulsion capacity and stability.

The lowest gelation concentration, water absorption and foaming properties were determined using the methods of Sathe *et al.* (1982), replacing water with appropriate salt solutions. The results were means of at least two determinations.

Statistical analysis. The statistical analysis used for determining differences between different salt concentrations (intra-salt solutions) were grand mean (\bar{X}), standard deviation (SD) and percent coefficient of variation (CV%). Rates were also determined for emulsion stability in addition to the above calculations (Steel and Torrie, 1960). The pH effects and the salt concentration effects are depicted in Fig. 1 and 2.

Results and Discussion

The proximate composition of *A. infracta* is shown in Table 1. The crude protein and carbohydrates were high with respective values of 27.79 ± 0.83 g/100 g and 47.22 ± 0.25 g/100 g. High value of the carbohydrates might be due to the incompletely digested leaves consumed by the silkworm larvae. While the protein value was higher than a typical legume seed like African yam bean (Adeyeye and Aye, 1998), the carbohydrate value was less than the carbohydrate values of the seeds under reference. The ash content 2.77 ± 0.33 g/100 g was lower than the value in grasshopper (*Zonocerus variegatus*) whose value was 3.1 ± 0.33 g/100 g (Olaofe *et al.*, 1998). The crude fat value of *A. infracta* was 9.16 ± 0.07 g/100 g. The protein and fat contents of locusts were 49-61 and 10-18%, respectively, and those of termites, 45 and 35.2%, respectively (Mayhew and Penny, 1988), and 54.9 and 13.3%, respectively, in *Z. variegatus* (Olaofe *et al.*, 1998). The proximate values, in the shell and flesh of three prawns from the Lagos lagoon were: crude protein (41.74-86.79%), ash (5.80-32.03%), fibre (0-18.84%) and carbohydrate (0.71-4.63%) (Adeyeye and Adubiaro, 2004).

The mineral composition is also given in Table 1. The iron content (16.02 ± 0.04 mg/100 g) was higher than that in various West African edible snails (4.6 - 9.3 mg/100 g) (Adeyeye, 1996a), freshwater fish (0.2-0.5 mg/100 g) (Adeyeye, 1994) and in *Z. variegatus* (3.7 ± 0.4 mg/100 g) (Olaofe *et al.*, 1998). The zinc

content is greater than that in the three snails cited above. *A. infracta* is a good source of magnesium, sodium and potassium. The sodium content (31.48 mg/100 g) was within the range of 12.5-63.1 mg/100 g reported for Nigerian freshwater fish (Adeyeye *et al.*, 1996) and close to the value of 36.6 mg/100 g in *Z. variegatus*, but the potassium value (36.74 mg/100 g) was greater than the range (12.5-16.9 mg/100 g) in the Nigerian freshwater fish and less than the value of 45.0 mg/100 g in *Z. variegatus*. The Na:K ratio was 1:1.2 or 31.4 mg/100 g: 36.74 mg/100 g; the balanced ratio of Na:K in the sample may not promote the chance of blood pressure in the consumers. The calcium content (26.48 mg/100 g) was within the range of that for snails 22.2-212 mg/100 g (Adeyeye, 1996a) but higher than *Illisha africana* fish (1.38-1.81 mg/100 g) (Adeyeye, 1996b) and *Z. variegatus* (8.4 mg/100 g). The minerals, not detected were: nickel, cobalt, lead and chromium though these were observed in some Nigerian snails (Adeyeye, 1996a). Nickel, cobalt and copper were all detected in shell and flesh of prawns in Lagos lagoon (Adeyeye and Adubiaro, 2004). The absence of lead was an indication that the environment might not have been contaminated by it. The value of manganese was low (0.07 mg/100 g) as in the snails (0.38 - 0.59 mg/100 g). Meat and poultry products contribute a little of this micro-mineral (Fleck, 1976) as observed in the present study. The phosphorus value (4110.71 mg/100 g) was higher than the

Table 1. Chemical composition of *Anaphe infracta*

Parameter	Concentration
Total ash ^a	2.77±0.33
Moisture ^a	10.65±0.71
Crude protein ^a	27.79±0.83
Carbohydrate ^a	47.22±0.25
Crude fat ^a	9.16±0.07
Fibre content ^a	2.91±0.03
Iron ^b	16.02±0.04
Zinc ^b	10.76±0.1
Copper ^b	ND
Sodium ^b	31.48±0.0
Nickel ^b	ND
Potassium ^b	36.74±0.1
Calcium ^b	26.48±0.1
Lead ^b	ND
Magnesium ^b	41.48±0.2
Manganese ^b	0.07±0.1
Cobalt ^b	ND
Phosphorus ^b	4110.71±0.2
Chromium ^b	ND

^a = g/100 g dry weight basis; ^b = mg/100 g dry weight basis; ND = not detected

values in snails (0.64-215.48 mg/100 g) (Adeyeye, 1996a), in prawns (660.0-1992.0 mg/100 g) (Adeyeye, 2000) and in the shell and the flesh of prawns of Lagos lagoon (699-2128 mg/100 g) (Adeyeye and Adubiaro, 2004) but slightly comparable to the values in fresh water crabs in Nigeria (350.55-2369.88 mg/100 g) (Adeyeye, 2002).

The values for the lowest gelation concentration are shown in Table 2 for all the salts. The salt free value was 8.0% while values of various salt concentrations ranged from 8.0-14.0% (NaCl), 10.0-14.0% (NaNO₃), 8.0-14.0% (Na₂CO₃), 6.0-8.0% (Na₂SO₃) and 8.0-12.0% (CH₃COONa) showing that the best salts to obtain the lowest gelation were Na₂SO₃ and CH₃COONa while the best salt concentrations were 0.5% w/v and 1.0% (w/v) in all the salts used. Most of these values were either lower or within the range or slightly higher than the values quoted for leguminous seeds (Adeyeye and Aye, 1998).

Variation in the gelling properties of the sample under different salt concentrations and anions might be due to their different effects on the relative ratios of different constituents: proteins, lipids and carbohydrates (Sathe *et al.*, 1982). The low values for the lowest gelation concentration (LGC) of *A. infracta* flour might likely lead to good setting of stews prepared from it. The percent coefficient of variation (CV%) of LGC ranged between 9.2-23.8 among various salts but ranged between 10.6-27.1 among salt concentrations; both the range of values were close.

No values were obtained for both foaming capacity and stability. This might be due to high denaturation of the sample flour.

Values for the water absorption capacity (WAC) are shown in Table 3. The value of WAC in distilled water was 144.5% but

ranged between 90.7-199.2% in various salt solutions. All the values of CV% were low. The best salt in this respect was NaNO₃ particularly at salt concentrations of 10.0% w/v and 15.0% w/v. The values compared favourably with WAC of 138% reported for pigeon pea flour (Oshodi and Ekperigin, 1989), 130% for soya flour and 107.1% for sunflower (Lin *et al.*, 1974), 127.5% for *Z. variegatus* (Olaofe *et al.*, 1998), but lower than the values reported for three varieties of melon by Ige *et al.* (1984) in the range of 200.0-288.8%. This means, *A. infracta* could be a useful replacement in food formulations such as soups or baked goods.

The oil emulsion capacity (OEC) varied from 0.0% in NaCl at all concentrations to 44.0% in CH₃COONa at 0.5% w/v salt concentration. The results in Table 4 show that oil emulsion capacity depends mostly on salt concentrations and the type of salt under consideration; CH₃COONa and Na₂SO₃ favoured emulsion formation while NaCl discouraged it. It is also evident that the CV% were highly varied at both the horizontal and the vertical levels. However, the OEC was better than the 7.0-11.0% reported for wheat flour and 18.0% for soya flour (Lin *et al.*, 1974) and 25.6% reported for *Z. variegatus* (Olaofe *et al.*, 1998). So *A. infracta* might be useful in the products such as sausages, soups and cakes (Altschul and Wilcke, 1985).

Values for the oil emulsion stability (OES) for *A. infracta* as a function of NaCl, NaNO₃, Na₂SO₃, Na₂CO₃ and CH₃COONa between 0.5% - 20.0% salt concentrations and stability period of 24 h are given in Table 5-9.

The capacity of protein to aid the formation and stabilization of emulsions is important for many applications in cake batter,

Table 2. Lowest gelation concentration (% dry weight) of *A. infracta* in various salt concentrations

Concentration of salt in distilled water (%)	Lowest gelation concentration (%)					\bar{X}	SD	CV(%)
	NaCl	NaNO ₃	Na ₂ CO ₃	Na ₂ SO ₃	CH ₃ COONa			
0.0	8.0	8.0	8.0	8.0	8.0	8.0	0.0	0.0
0.5	8.0	10.0	8.0	8.0	8.0	8.4	0.9	10.6
1.0	10.0	12.0	10.0	6.0	8.0	9.2	2.3	24.8
2.0	10.0	12.0	12.0	8.0	12.0	10.8	1.8	16.6
5.0	10.0	12.0	12.0	8.0	8.0	10.0	2.0	20.0
10.0	14.0	14.0	12.0	8.0	8.0	11.2	3.0	27.1
15.0	14.0	14.0	12.0	8.0	10.0	11.6	2.6	22.5
20.0	14.0	14.0	14.0	8.0	12.0	12.4	2.6	21.1
Mean (\bar{X})	11.0	12.0	11.0	7.8	9.3	-	-	-
SD	2.6	2.1	2.1	0.7	1.8	-	-	-
CV %	23.8	17.8	19.5	9.2	19.8	-	-	-

SD = standard deviation; CV = coefficient of variation

Table 3. Water absorption capacity of *A. infracta* in various salt concentrations

Concentration of salt in distilled water (%)	Water absorption capacity (%)					\bar{X}	SD	CV(%)
	NaCl	NaNO ₃	Na ₂ CO ₃	Na ₂ SO ₃	CH ₃ COONa			
0.0	144.5	144.5	144.5	144.5	144.5	144.5	0.0	0.0
0.5	135.4	149.3	170.9	166.5	161.5	156.7	14.4	9.2
1.0	166.5	150.4	157.7	166.6	154.0	159.0	7.4	4.6
2.0	159.9	163.7	159.4	170.2	153.2	161.3	6.2	3.9
5.0	146.1	184.4	163.4	166.8	149.7	162.1	15.3	9.4
10.0	151.4	199.2	150.2	161.2	154.9	165.5	23.0	13.9
15.0	149.3	198.8	123.3	153.0	143.0	153.5	27.8	18.1
20.0	149.1	174.7	90.7	94.1	145.2	130.8	36.9	28.2
Mean (\bar{X})	150.3	170.6	145.0	152.8	150.7	-	-	-
SD	9.5	22.1	26.3	25.3	6.3	-	-	-
CV	6.3	13.0	18.1	16.5	4.2	-	-	-

SD = standard deviation; CV = coefficient of variation

Table 4. Oil emulsion capacity of *A. infracta* in various salt concentrations

Concentration of salt in distilled water (%)	Oil emulsion capacity (%)					\bar{X}	SD	CV(%)
	NaCl	NaNO ₃	Na ₂ SO ₃	Na ₂ CO ₃	CH ₃ COONa			
0.0	20.0	20.0	20.0	20.0	20.0	20.0	0.0	0.0
0.5	0.0	20.0	20.0	20.0	44.0	26.0	12.0	46.2
1.0	0.0	0.0	20.0	12.0	40.0	18.0	16.8	93.4
2.0	0.0	20.0	20.0	12.0	20.0	18.0	4.0	22.2
5.0	0.0	20.0	20.0	0.0	20.0	15.0	10.0	66.7
10.0	0.0	20.0	20.0	20.0	20.0	20.0	0.0	0.0
15.0	0.0	0.0	40.0	40.0	20.0	25.0	19.2	76.6
20.0	0.0	0.0	20.0	20.0	20.0	15.0	10.0	66.7
Mean (\bar{X})	ND	12.5	22.5	18.0	25.5	-	-	-
SD	-	10.4	7.1	11.3	10.2	-	-	-
CV	-	82.8	31.4	62.8	40.2	-	-	-

SD = standard deviation; CV = coefficient of variation; * = horizontal values of \bar{X} , SD and CV (%) exclude values for NaCl; ND = not determined

coffee whitener, milk, mayonnaise, salad dressings, comminuted meats and frozen desserts (Kinsella *et al.*, 1985). The OES values were best in Na₂SO₃, NaCl, NaNO₃ (in decreasing order) at the salt concentrations of 0.5% - 2.0% w/v but stability values for those salts were less at salt concentrations between 5.0% - 20.0% w/v. Lower values of OES were observed for CH₃COONa and Na₂CO₃ (in the decreasing order) but in a reverse situation, the salt concentrations of Na₂CO₃ at 5.0% - 20.0% w/v recorded high OES values. The enhanced OES for some of the salt concentrations might be due to more exposure of the oil binding domain.

The change in OES, with the increase of time (as shown in the CV%), was low in NaCl (0.0-3.1%), NaNO₃ (0.0-2.3%), Na₂SO₃ (0.0- 4.3%) and CH₃COONa (0.0-3.9%), while the change was

relatively high in Na₂CO₃ (1.9-41.4%). Also the rate of change was low in many salts: NaCl (0.0-0.12 cc/h), NaNO₃ (0.0-0.06 cc/h) and CH₃COONa (0.0-0.07 cc/h) but was relatively higher in Na₂CO₃ (0.06-0.33 cc/h). This meant that Na₂CO₃ was the least stable. The decrease in emulsion stability with the increase of time might be due to increased contact leading to coalescence thereby reducing stability (Parker, 1987). Most of the values for the rate of change here were better than the values reported for many varieties of African yam bean (AYB) seeds whose values ranged between 0.98-1.60 cc/h (Adeyeye and Aye, 1998). This meant that *A. infracta* might be a better oil emulsion stabilizer than AYB.

Effect of pH on the protein solubility of *A. infracta* is depicted in Fig. 1. The sample flour showed maximum protein solubility

Table 5. Oil emulsion stability (cc) of *A. infracta* as a function of NaCl

Time (h)	Concentration of NaCl in distilled water (%)							\bar{X}	SD	CV(%)
	0.5	1.0	2.0	5.0	10.0	15.0	20.0			
0.0	36.7	34.7	37.8	31.9	31.9	31.2	33.8	34.0	2.6	7.6
1.0	36.7	34.7	37.8	31.9	31.9	31.2	33.8	34.0	2.6	7.6
2.0	35.3	34.7	37.8	31.9	31.9	30.4	33.8	36.7	2.5	6.9
3.0	35.3	34.7	37.8	31.9	31.9	30.4	33.8	33.7	2.5	7.5
4.0	35.3	34.7	37.8	31.9	31.9	30.4	33.8	33.7	2.5	7.5
5.0	35.3	34.7	37.8	31.9	31.9	30.4	33.8	33.7	2.5	7.5
22.0	33.8	34.7	36.5	30.4	31.9	30.4	33.8	33.1	2.3	6.9
24.0	33.8	34.7	36.5	30.4	31.9	30.4	33.8	33.1	2.3	6.9
\bar{X}	35.3	34.7	37.5	31.5	31.9	30.6	33.8	-	-	-
SD	1.1	0.0	0.6	0.7	0.0	0.3	0.0	-	-	-
CV(%)	3.1	0.0	1.7	2.1	0.0	1.1	0.0	-	-	-
Rate of change (cc/h)	0.12	0.0	0.06	0.06	0.0	0.03	0.00	-	-	-

\bar{X} = mean; SD = standard deviation; CV = coefficient of variation

Table 6. Oil emulsion stability (cc) of *A. infracta* as a function of NaNO₃

Time (h)	Concentration of NaNO ₃ in distilled water (%)							\bar{X}	SD	CV(%)
	0.5	1.0	2.0	5.0	10.0	15.0	20.0			
0.0	33.0	33.3	33.3	34.3	33.3	30.4	33.3	33.0	1.2	3.6
1.0	33.0	33.3	33.3	34.3	33.3	30.4	33.3	33.0	1.2	3.6
2.0	33.0	31.8	33.3	34.3	33.3	30.4	33.3	32.8	1.3	3.9
3.0	33.0	31.8	33.3	34.3	33.3	30.4	33.3	32.8	1.3	3.9
4.0	33.0	31.8	31.9	32.9	33.3	30.4	33.3	32.4	1.1	3.3
5.0	33.0	31.8	31.9	32.9	31.9	30.4	31.9	32.0	0.8	2.6
22.0	33.0	31.8	31.9	32.9	31.9	30.4	31.9	32.0	0.8	2.6
24.0	33.0	31.8	31.9	32.9	31.9	30.4	31.9	32.0	0.8	2.6
\bar{X}	33.0	32.2	32.6	33.6	32.8	30.4	32.8	-	-	-
SD	0.0	0.7	0.8	0.8	0.7	0.0	0.7	-	-	-
CV(%)	0.0	2.1	2.3	2.3	2.1	0.0	2.1	-	-	-
Rate of change (cc/h)	0.0	0.06	0.06	0.06	0.06	0.0	0.06	-	-	-

\bar{X} = mean; SD = standard deviation; CV = coefficient of variation

in both acidic and basic regions of the pH scale. The isoelectric point (IEP) at pH 5 was recorded to be 6.87%. Highest solubility in the acidic region was 10.31% (pH 4) while it was 9.84% (pH 10) at basic region. This meant that the *A. infracta* flour might be useful in the formulation of acid foods such as protein rich carbonated beverages and milk analogue products (Cherry, 1981; Kinsella, 1979). However the solubility was generally low, which might be due to heat denaturation during sample preparation. Reduction in protein solubility due to heat processing has been reported in the case of soya bean

and peanut flours (McWatters and Holmes, 1979) and also cowpea flour protein (Abbey and Ayuh, 1991). The *A. infracta* solubility might be improved by using less drastic heating for the sample preparation.

The effect of concentration on the protein solubility of *A. infracta* is shown in Fig. 2. The minimum solubilities (IEP) were recorded for various salts at various concentrations, viz; NaCl, 6.3% (8.1×10^{-3} M or 5.0%); Na₂CO₃, 9.4% (1.5×10^{-2} M or 20.0%); NaNO₃, 3.1% (1.9×10^{-2} M or 20.0%); Na₂SO₃, 1.6% (7.1×10^{-3} M or 10.0%) and CH₃COONa, 6.6% (1.6×10^{-2} M

Table 7. Oil emulsion stability (cc) of *A. infracta* as a function of Na₂SO₃

Time (h)	Concentration of Na ₂ SO ₃ in distilled water (%)							\bar{X}	SD	CV(%)
	0.5	1.0	2.0	5.0	10.0	15.0	20.0			
0.0	36.3	39.2	37.3	33.8	34.8	33.3	34.3	35.6	2.1	6.0
1.0	32.3	39.2	37.3	33.8	34.8	33.3	34.3	35.0	2.4	6.9
2.0	32.3	39.2	37.3	32.4	34.8	33.3	34.3	34.8	2.6	7.4
3.0	32.3	39.2	37.3	32.4	33.3	33.3	34.3	34.6	2.6	7.6
4.0	32.3	39.2	37.3	32.4	33.3	33.3	34.3	34.6	2.6	7.6
5.0	32.3	37.8	37.3	32.4	33.3	33.3	32.8	34.2	2.4	6.9
22.0	32.3	37.8	37.3	32.4	33.3	33.3	32.8	34.2	2.4	6.9
24.0	32.3	37.8	37.3	32.4	33.3	33.3	32.8	34.2	2.4	6.9
\bar{X}	22.8	38.7	37.3	32.7	33.9	33.3	33.8	-	-	-
SD	1.4	0.7	0.0	0.7	0.8	0.0	0.8	-	-	-
CV(%)	4.3	1.8	0.0	2.0	2.2	0.0	2.3	-	-	-
Rate of change (cc/h)	0.17	0.06	0.0	0.06	0.06	0.0	0.06	-	-	-

\bar{X} = mean; SD = standard deviation; CV = coefficient of variation

Table 8. Oil emulsion stability (cc) of *A. infracta* as a function of Na₂CO₃

Time (h)	Concentration of Na ₂ CO ₃ in distilled water (%)							\bar{X}	SD	CV(%)
	0.5	1.0	2.0	5.0	10.0	15.0	20.0			
0.0	5.6	6.8	9.2	45.3	37.8	39.2	41.1	26.4	18.2	68.7
1.0	5.6	6.8	9.2	45.3	37.8	39.2	41.1	26.4	18.2	68.7
2.0	5.6	6.8	9.2	40.0	37.8	39.2	39.7	25.5	17.2	67.3
3.0	5.6	6.8	9.2	38.7	36.5	39.2	39.7	25.1	16.8	66.9
4.0	5.6	6.8	9.2	37.3	36.5	36.5	38.4	24.3	16.1	66.1
5.0	4.2	5.4	6.6	37.3	36.5	36.5	37.0	23.4	16.8	72.0
22.0	2.8	2.7	2.6	37.3	36.5	36.5	37.0	22.2	18.2	82.1
24.0	2.8	2.7	2.6	37.3	36.5	36.5	37.0	22.2	18.2	82.1
\bar{X}	4.8	5.6	7.2	39.8	37.0	37.8	38.9	-	-	-
SD	1.3	1.8	3.0	3.5	0.7	1.4	1.8	-	-	-
CV(%)	27.2	33.0	41.4	8.8	1.9	3.8	4.6	-	-	-
Rate of change (cc/h)	0.12	0.17	0.27	0.33	0.06	0.11	0.17	-	-	-

\bar{X} = mean; SD = standard deviation; CV = coefficient of variation

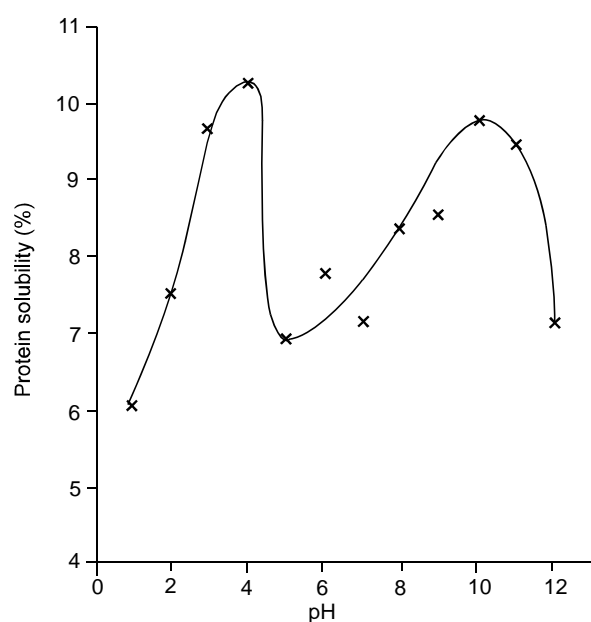
or 15.0% w/v). Maximum solubility values also varied similarly *viz*: NaCl, 17.2% (8.5×10^{-4} M or 0.5%); Na₂CO₃, 29.7% (4.5×10^{-3} M or 5.0%); NaNO₃, 18.8% (2.3×10^{-3} M or 2.0%); Na₂SO₃, 7.8% (7.9×10^{-4} M or 1.0%) and CH₃COONa, 19.4% (2.4×10^{-3} M or 2.0% w/v). The lyotropic series is therefore, in the order: CO₃²⁻ > CH₃COO⁻ > NO₃⁻ > Cl⁻ > SO₃²⁻. Shen (1981) studied the effect of various neutral salts on the solubility of soya protein and reported low solubility of protein at low concentrations of 0.2 M - 0.3 M but higher solubilities at higher salt concentrations. This observation was noted in some of the salts (Fig. 2). The initial decrease in solubility was due to

electrostatic shielding of the charged groups by salts, which decreases the electrostatic repulsion and apparently enhances the hydrophobic interactions. This is supported by the fact that in many proteins the electrostatic interactions are essentially suppressed at 0.15 M ionic strength (Eagland, 1975). In the case of CO₃²⁻, the solubility increased above 0.5% w/v or 1.4×10^{-3} ionic strength, (Fig. 2), indicating that the hydrophobic interactions between the molecules were suppressed. However, in the case of SO₃²⁻, which is a water-structure-enhancing ion (Kinsella *et al.*, 1985), the solubility was low in virtually all the concentrations indicating that SO₃²⁻ has only

Table 9. Oil emulsion stability (cc) of *A. infracta* as a function of CH₃COONa

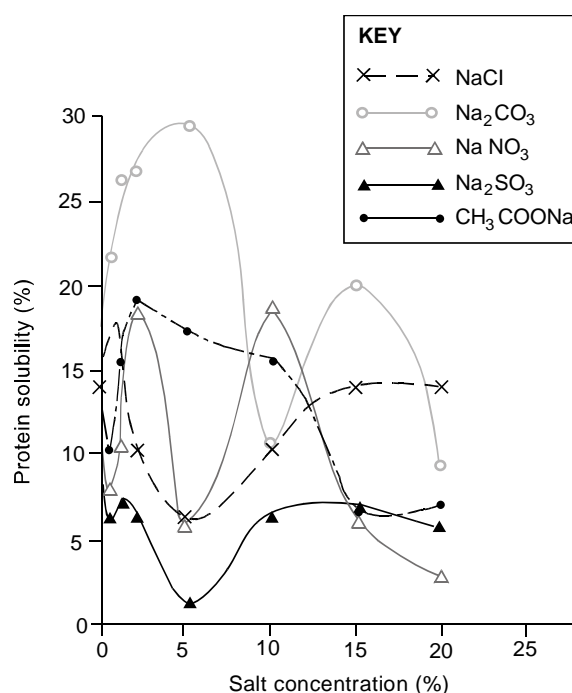
Time (h)	Concentration of CH ₃ COONa in distilled water (%)							\bar{X}	SD	CV(%)
	0.5	1.0	2.0	5.0	10.0	15.0	20.0			
0.0	26.3	21.3	23.0	23.8	24.2	22.6	24.6	23.7	1.6	6.8
1.0	26.3	21.3	23.0	23.8	24.2	22.6	24.6	23.7	1.6	6.8
2.0	26.3	21.3	23.0	23.8	24.2	22.6	24.6	23.7	1.6	6.8
3.0	26.3	21.3	21.3	23.8	24.2	22.6	24.6	23.6	1.8	7.8
4.0	24.6	21.3	21.3	23.8	24.2	22.6	24.6	23.2	1.5	6.3
5.0	24.6	21.3	21.3	23.8	22.6	22.6	24.6	23.0	1.4	6.1
22.0	24.6	21.3	21.3	23.8	22.6	22.6	24.6	23.0	1.4	6.1
24.0	24.6	21.3	21.3	23.8	22.6	22.6	24.6	23.0	1.4	6.1
\bar{X}	25.4	21.3	21.9	23.8	23.6	22.6	24.6	-	-	-
SD	0.9	0.0	0.9	0.0	0.8	0.0	0.0	-	-	-
CV(%)	3.7	0.0	3.9	0.0	3.5	0.0	0.0	-	-	-
Rate of change (cc/h)	0.07	0.0	0.07	0.0	0.07	0.0	0.0	-	-	-

\bar{X} = mean; SD = standard deviation; CV = coefficient of variation

**Fig. 1.** Effect of pH on the protein solubility of *A. infracta*.

positive effect on the hydrophobic interactions. Table 10 depicts various salts concentrations (percentage and molarity) and ionic strength (μ).

Globulin solubility is dependent upon salt concentration. The effect of salts in increasing the solubility of globulins is called "salting-in" effect. The solubility is a function of the ionic strength, which is readily calculated from the molar concentrations of the ions and their charge, using the expression:

**Fig. 2.** Effect of salts concentration on the protein solubility of *A. infracta*.

$$\mu = \frac{1}{2} \sum m Z^2$$

where:

μ is the ionic strength, m the molarity and Z the charge of the ion; Σ denotes that the mZ^2 terms are added for each of the ions (White *et al.*, 1973) (Table 10).

Table 10. Various salts concentrations (percentage and molarity) and ionic strength (μ)

Salt	Percentage concentration	Molarity	Ionic strength
NaCl	0.5	8.5×10^{-4}	8.5×10^{-4}
NaCl	1.0	1.7×10^{-3}	1.7×10^{-3}
NaCl	2.0	3.4×10^{-3}	3.4×10^{-3}
NaCl	5.0	8.1×10^{-3}	8.1×10^{-3}
NaCl	10.0	1.5×10^{-2}	1.5×10^{-2}
NaCl	15.0	2.2×10^{-2}	2.2×10^{-2}
NaCl	20.0	2.7×10^{-2}	2.7×10^{-2}
NaNO ₃	0.5	5.9×10^{-4}	5.9×10^{-4}
NaNO ₃	1.0	1.2×10^{-3}	1.2×10^{-3}
NaNO ₃	2.0	2.3×10^{-3}	2.3×10^{-3}
NaNO ₃	5.0	5.6×10^{-3}	5.6×10^{-3}
NaNO ₃	10.0	1.1×10^{-2}	1.1×10^{-2}
NaNO ₃	15.0	1.5×10^{-2}	1.5×10^{-2}
NaNO ₃	20.0	1.9×10^{-2}	1.9×10^{-2}
Na ₂ SO ₃	0.5	3.9×10^{-4}	1.2×10^{-3}
Na ₂ SO ₃	1.0	7.9×10^{-4}	2.4×10^{-3}
Na ₂ SO ₃	2.0	1.6×10^{-3}	4.8×10^{-3}
Na ₂ SO ₃	5.0	3.8×10^{-3}	1.14×10^{-2}
Na ₂ SO ₃	10.0	7.1×10^{-3}	2.14×10^{-2}
Na ₂ SO ₃	15.0	1.01×10^{-2}	3.03×10^{-2}
Na ₂ SO ₃	20.0	1.3×10^{-2}	3.9×10^{-2}
Na ₂ CO ₃	0.5	4.7×10^{-4}	1.4×10^{-3}
Na ₂ CO ₃	1.0	9.3×10^{-4}	2.8×10^{-3}
Na ₂ CO ₃	2.0	1.8×10^{-3}	5.4×10^{-3}
Na ₂ CO ₃	5.0	4.5×10^{-3}	1.35×10^{-2}
Na ₂ CO ₃	10.0	8.5×10^{-3}	2.6×10^{-2}
Na ₂ CO ₃	15.0	1.2×10^{-2}	3.6×10^{-2}
Na ₂ CO ₃	20.0	1.5×10^{-2}	4.5×10^{-1}
CH ₃ COONa	0.5	6.1×10^{-4}	6.1×10^{-4}
CH ₃ COONa	1.0	1.2×10^{-3}	1.2×10^{-3}
CH ₃ COONa	2.0	2.4×10^{-3}	2.4×10^{-3}
CH ₃ COONa	5.0	5.8×10^{-3}	5.8×10^{-3}
CH ₃ COONa	10.0	1.1×10^{-2}	1.1×10^{-2}
CH ₃ COONa	15.0	1.6×10^{-2}	1.6×10^{-2}
CH ₃ COONa	20.0	1.95×10^{-2}	1.95×10^{-2}

The solubility in most of the salt solutions was better than in pH. However, there was general decrease in solubility for most of the salts particularly at concentrations 15.0-20.0% w/v which might be due to denaturation. The graphs for effects of pH and salts (Fig. 1 and 2) show at least two distinct peaks indicating that *A. infracta* might be having two distinct major proteins.

In conclusion, it can be said that *A. infracta* is a good source of both major and minor minerals with a good Na-K ratio and

high protein content. The following functional properties are also favourable: lowest gelation concentration, water absorption capacity, oil emulsion capacity, oil emulsion stability and protein solubility, making it potentially useful in some food formulations.

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