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Effect of NaCl and its partial or complete replacement with KCl on some functional properties of defatted *Colocynthis citrullus* L. seed flour

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Abstract

Effect of NaCl on some hydration and hydration related properties of *Colocynthis citrullus* L. were investigated together with the possibility of replacing such salt with KCl in food formulation. This study showed that protein solubility increased with increase in salt concentration. Partial replacement of NaCl with KCl did not make any significant difference to protein solubility. Water absorption capacity decreased with increase in salt concentration and neither complete nor partial replacement of NaCl with KCl made any significant difference ($P > 0.05$). Foaming capacity of *C. citrullus* was improved at low NaCl and KCl concentration ranges (0.25–2.00% NaCl; 0.25–1.75% KCl). Partial replacement caused little or no improvement in foaming attributes. The least gelation concentration was 14% (w/v) and improved to 4% (w/v) and 2% (w/v) in NaCl and NaCl & KCl (mixture) solutions, respectively; KCl solution had no effect.

1. Introduction

Studies of plant protein, as a non-conventional protein source, have been on the increase, due to the new challenge of providing adequate protein for an expanding world population. Also, exploiting such resources can boost economic development, especially in the developing countries, where protein intake is less than desirable (Satterlee, 1981). Increased cultivation of *Colocynthis citrullus* L, that belongs to the family of cucurbitaceae, has been advocated because of its dietary importance, especially where protein is deficient in normal food (Oyolu, 1977). Even though the biological value, net protein utilization, and protein efficiency ratio of *C. citrullus* L. seed flour products are lower than values obtained for soyabean, they are comparable to or higher than most oil seeds (Oyenuga & Fetuga, 1975; Umoh & Oke, 1974). *C. citullus* L. is used in several dietary preparations; its whole seed can be roasted and consumed as snacks; defatted seed meals can also be made into patties and serve as meat substitute. Its seed flour contains several macronutrients that could contribute significantly to diets. Its good potential in a number of food formulations has been suggested especially in the low milk-consuming regions of West Africa where its cultivation thrives (Akobundu, Cherry, & Simmon, 1982).

Food application of *C. citrullus* L. vegetable, as a protein source or functional ingredient, depends on the knowledge of its functional properties and the factors, which influence functionalities, i.e. intrinsic, environmental and process treatment factors (Pomeranz, 1991; Nakai & Powrie, 1981). Factors, such as pH and salts, affect physicochemical properties and interaction between proteins and in turn alter functional properties (Kinsella, 1979; Mwasaru, Muhammed, Bakar, & Che-Man, 2000; Philips, Yang, & Kinsella, 1991). NaCl is a principal ingredient in food formulation due to its flavour, preservative and protein-solubilizing properties (Gimeno, Astiasaran, & Bello, 1999; Seman, Olson, & Mandigo, 1980). However, total or partial substitution of NaCl with other plausible salts is an aim of food industries, to reduce the sodium content of foods, since

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excessive intake of sodium (> 3.5 g NaCl/day) is associated with hypertension. Patients suffering from coronary heart disease and related symptoms are generally placed on a severely restricted Na intake of 200 mg per day (Gimeno et al., 1999; Hand, Terrel, & Smith, 1982a,b). The use of KCl, despite its less agreeable taste has been suggested (Ranken & Kill, 1993).

The chemical composition and functional properties of *C. citrullus* have been investigated (Ige, 1984; King & Onuora, 1984; Oyolu, 1977; Oyolu & Macfalane, 1982); however, a literature search showed that the effect of salt on its functional properties has not been reported. This study, therefore aimed at investigating the effect of NaCl on hydration-related properties of its seed flour. Studies on the possibilities of partially or completely replacing NaCl with KCl were also undertaken, to further project the properties of *C. citrillus* and its potential in practical food applications.

2. Materials and methods

2.1. Material

Dehulled *Colocynthis citrullus* L. seeds were purchased from local markets at Abeokuta in Ogun State of Nigeria. The seeds were screened, milled and defatted by multiple extractions with cold acetone. The defatted sample was air dried at room temperature (30 °C) for about 24 h, reground to pass through a 50 mesh stainless steel sieve and stored for use as defatted *Colocynthis citrullus* L. seed flour (DCCF).

2.2. Protein solubility

Protein solubility (PS) was determined for the distilled water-flour and salt solution-flour suspensions by the method described by Padilla, Alvarez and Alfaro (1996), where 0.5 g of the flour was suspended in 50 ml distilled water, the suspension stirred on an orbital shaker (Stwart Scientific 501) at room temperature for 20 min and centrifuged at 10,000 rpm for 10 min. Clear supernatant was obtained; protein content was determined by the Biuret method (Gornall, Bardawill, & David 1949). The distilled water was later replaced with appropriate salt solution. The salts used were NaCl, KCl and NaCl & KCl mixture at concentrations 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 3.00, 4.00 and 5.00%. The solution of the NaCl & KCl mixture was obtained by mixing 25 ml of each salt solution at their respective concentrations. In all the determinations, the suspensions were maintained at their "natural pH" (reconstituted pH of flour suspension which is between pH 6.1 and 6.8). The "natural pH" was chosen, this pH is likely observed when the seed flour is reconstituted in either water or salt solution prior to use (Sze-Tao & Sathe, 2000).

2.3. Water absorption capacity

Two grams of the DCCF were suspended in 20 ml distilled water and kept at a room temperature for $1^1/_2$ h (Okaka & Potter, 1979). The suspension was centrifuged at 5000 rpm for 15 min. The distilled water was later replaced by salts at various concentrations. The amount of water absorbed by the DCCF was determined by the difference and defined as the absorption capacity.

2.4. Foaming capacity and stability

One and a half grams of DCCF were suspended in 50 ml distilled water within the "natural pH" ranges indicated above; the suspensions were stirred in a Moulinex blender for 3 min (Akintayo, Oshodi, & Esuoso, 1999). The content was transferred into a 250 ml-measuring cylinder and the volume of foam was read after 30 s for foam capacity (FC). This was repeated for the various salt solutions at different concentrations, as discussed above. The foam stability (FS) was determined by measuring the volume of foam at 10, 30, 60 and 120 min, after pouring the whipped suspension. The percentage ratio of the volume increase (to that of the original volume of protein solution) was calculated and expressed as foaming capacity (Naczk, Prosady, & Rubin, 1985). All determinations were carried out at least in duplicate.

2.5. Gelation capacity

Gelation capacity (GC) was determined according to the method of Coffman and Garcia (1977), as modified by Akintayo et al. (1999). Sample suspensions 2–20% (w/v) were prepared in 5 ml distilled water and salt solutions at various concentrations in test tubes. The suspensions were heated in a boiling water bath for 1 h followed by rapid cooling in a cold water-bath. The samples were further cooled at 5 ± 1 °C for 2 h. Results reported are means of duplicate determinations.

2.6. Statistical analysis

PS and WAC data were subjected to analysis of variance and the least square means compared using the general linear model (SAS, 1994).

3. Results and discussion

3.1. Protein solubility

Table 1, shows the effect of salt types, at varying concentrations, on solubility of DCCF protein. Generally, PS was observed to increase with increase in salt concentration; however the increase became statistically

Table 1
Effect of various salt solutions on protein solubility of DCCF[a]

Salt solution concentration (%, w/v)	Protein solubility (mg/ml)		
	NaCl	NaCl and KCl	KCl
0.00	0.353±0.042ab	0.340±0.020a	0.343±0.021a
0.25	0.550±0.026abc	0.557±0.015abc	0.347±0.029a
0.5	0.573±0.025abc	0.600±0.036abc	0.447±0.032ab
0.75	0.470±0.030ab	0.653±0.055abc	0.510±0.010ab
1.00	0.627±0.45abc	0.787±0.090abcd	0.607±0.045abc
1.50	0.753±0.006abc	0.897±0.058bcde	0.653±0.031abc
2.00	1.307±0.297def	1.077±0.065cde	0.687±0.050abc
3.00	1.773±0.364fg	1.997±0.038gh	1.023±0.059cde
4.00	2.480±0.062h	2.277±0.058gh	1.380±0.053ef
5.00	2.147±0.100gh	2.457±0.107h	2.057±0.096gh

[a] Results are means of triplicate determination. Values in either row or column with the same letters are not statistically different ($P<0.05$).

significant ($P<0.05$) only at concentrations 2.00, 3.00 and 4.00% in NaCl, NaCl and KCl & KCl salt solutions, respectively. In 5% NaCl solution, a slight decrease was observed. A similar observation was reported by Padilla et al. (1996), in which the solubility of *Caryodendron orinocense* Karst. flour protein increased with NaCl concentration up to 0.5 M, and thereafter decreased. This may be due to the low ionic strength of these salts, which allows dissociation and consequent interaction with the proteins, thereby increasing solubility (*'salting in' effect*). But at higher concentrations, NaCl produces a dehydrating effect on the protein, which tends to aggregate, resulting in decrease of solubility (*'salting out' effect*) (Padilla et. al., 1996).

In most of the salts considered at different concentrations, PS was not significantly different in the concentration range 0.25–1.50%, but within concentration range of 2–4%, protein solubility of DCCF is significantly lower in KCl compared to NaCl. Salts that are not able to effectively destabilize native globular and fibrous structure and thereby decrease the rate of their denaturation may increase solubility (Oshodi & Ojokan, 1997; Shen, 1981; Von-Hippel & Scheleich, 1969). It can then be concluded that neither NaCl nor KCl or NaCl & KCl mixture at the concentrations considered, are capable of actively increasing rates of conformational change of DCCF protein thus more protein, may be available in food formulations.

3.2. Water absorption capacity

Table 2 presents the results of water absorption capacity (WAC) of DCCF in different salt solutions. A consistent decrease in WAC, as salt concentrations increased, was observed, but the decrease became significant ($P<0.05$) in NaCl and KCl solutions at concentration $\geqslant 1.5\%$ while, in salt mixture (NaCl & KCl), it became significant from 1.0%. Oshodi and Ojokan (1997) also reported a decrease in WAC of bovine plasma protein concentrate in NaCl solution with increase in concentration ($\leqslant 5\%$). This trend may be due to masking of charges as concentration increased, which can reduce electrostatic interaction and hydration but increase hydrophobic interaction (Alschul & Wilcks, 1985; Kinsella, Damodaran, & German, 1985; Oshodi &

Table 2
Effect of various salt solutions on water absorption capacity of DCCF[b]

Salt solution concentration (%,w/v)	Water absorption capacities (%)		
	NaCl	NaCl and KCl	KCl
0.00	303±8ab	313±6a	307±15ab
0.25	295±13ab	285±23b	292±8b
0.50	310±5ba	290±15b	302±8ab
0.75	306±2ab	306±16ab	298±12ab
1.00	298±8ab	260±13c	305±5ab
1.50	257±8c	256±10c	258±10c
2.00	241±18cde	258±24c	250±15cd
3.00	230±17def	250±10cd	222±3efg
4.00	208±8gh	210±22fgh	218±10fgh
5.00	205±13gh	198±3h	211±8fgh

Ojokan, 1997; Shen, 1981). Though the WAC observed for DCCF decreased with increase in salt concentrations, the lowest WAC values observed at 5% concentration of various solutions, i.e. 211% of KCl; 205% of NaCl; 198% of NaCl and KCl mixture, are much higher than that of soya bean flour protein concentrate (112.43%), sun flour protein concentrate (137%) and *Adenopus breviflorus* Benth seed flour (175.0%) in distilled water (Lin, Humbert, & Sosulski, 1974; Oshodi, 1992; Padilla et al., 1996).

This result suggests that DCCF proteins may be more hydrophilic or that the flour contains more polysaccharide, which is capable of entrapping more water than proteins (Oshodi, 1992; Padilla et al., 1996). The high WAC reported in this study is a useful indication that DCCF will perform well in texture of comminuted meat and baked products (Okezie & Bello, 1988). Effect of salt type is not significant at $P<0.05$. Thus, for patients with coronary heart disease and low Na requirement, either partial or complete substitution of NaCl with KCl will not make any significant difference when DCCF is employed in food formulation.

3.3. Foam capacity and stability

The results showing the effect of NaCl, KCl and NaCl&KCl on DCCF foaming strength are presented in Fig. 1. NaCl and KCl solutions were found to improve the FC of DCCF, while little or no improvement was observed in NaCl & KCl solutions. This observed improvement of FC in NaCl and KCl solutions was, however, a function of salt concentration. In NaCl solution, improved foaming was observed within a concentration range of 0.25–2.0% while, in KCl solution, notable improvements were found in the range of 0.25–1.75% (except 1.0% KCl). The highest FC values of 28 and 20% were found in 0.75% KCl and 1.00% NaCl, respectively, foam depression was observed with further increase in salt concentration in each case.

According to Alschul and Wilke (1985), salts at appropriate concentrations reduce surface viscosity and rigidity of protein films but increase spreading rate, which in turn weakens interpeptide attraction, thereby aiding foaming for certain proteins. It therefore seemed reasonable to deduce that concentrations greater than 0.75% KCl and 1.00% NaCl do not have much effect on the rigidity and viscosity of DCCF globular protein.

The results of FS at 10, 30, 60 and 120 min, for various salts at different concentrations, are shown in Fig. 2–4. The stability of DCCF foam was enhanced at low KCl and NaCl concentrations. A significant enhancement in FS was observed in 0.75% KCl. The foam formed in NaCl & KCl mixture had almost completely collapsed after 2 h.

The improved FC and FS of DCCF in the presence of NaCl and KCl at appropriate concentration will enhance its functionality and thus its application in foods such as whipped toppings, cake-mixes and frosting (Kinsella, 1979; Ma & Harwalkar, 1984), where foaming is of paramount importance. Also where low Na intake is recommended, KCl can be a full substitute for NaCl rather than partial replacement of NaCl with KCl.

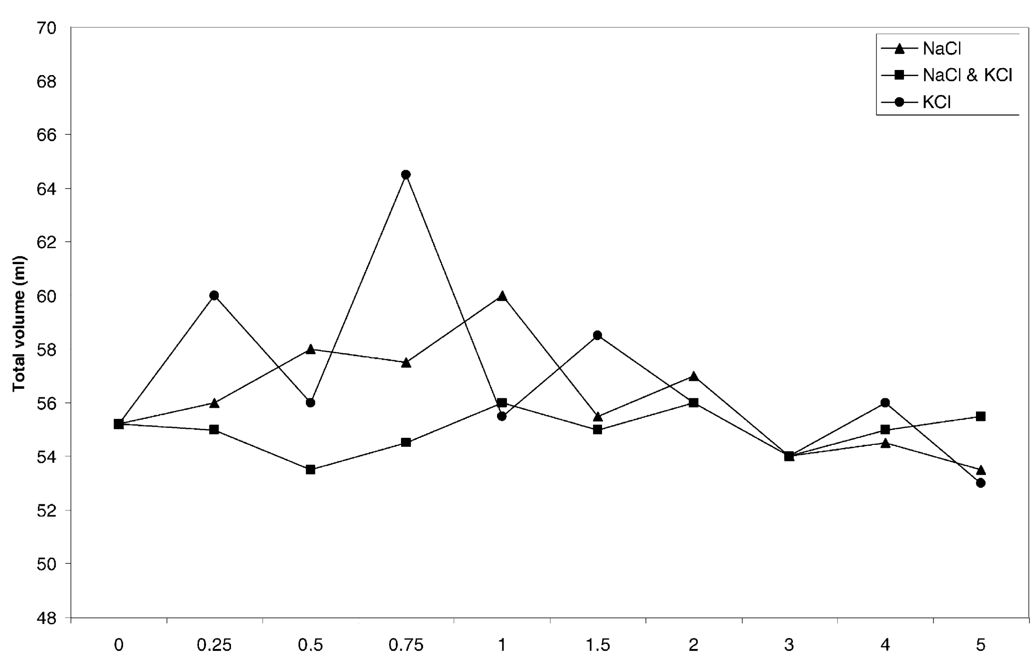

Fig. 1. Foaming capacity profile of DCCF at various salt concentrations.

3.4. Gelation capacity

The gelating capacity (GC) of DCCF (Fig. 5) was assessed by determining the least gelation concentration (LGC) which is operationally defined as the lowest concentration at which gel remains in the inverted tube The LGC of DCCF in distilled water is 14% (w/v), which is the same as for lupin seed (Sathe, Deshpande, & Salunkhe, 1982), *Adenopus breviflorus* (Oshodi, 1992) and *Caryodendron orinocense* (Padilla et al., 1996) but higher than those of Great Northern bean flour of 10% (w/v) (Sathe & Salunkhe, 1981) and pigeon pea of 12% (w/v) (Oshodi & Ekperigin, 1989). *Colocynthis* LGC was improved from 14% (w/v) to 4% (w/v) in the presence of 5% NaCl. A better improvement was observed on partial replacement of NaCl with KCl, where LGC was

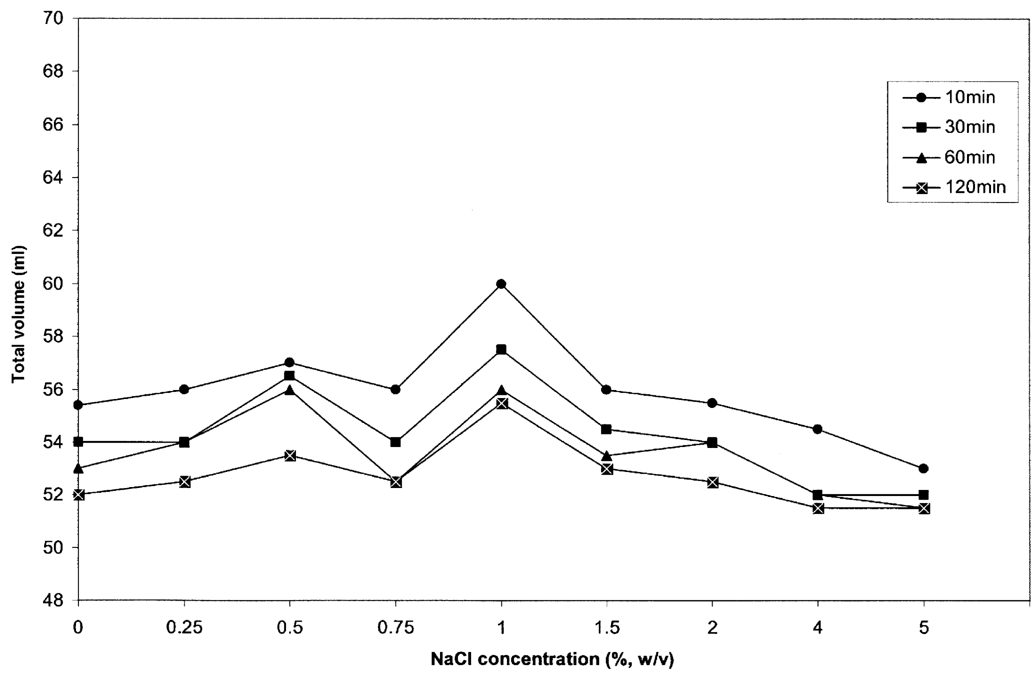

Fig. 2. Effect of NaCl at various concentrations on DCCF foaming stability.

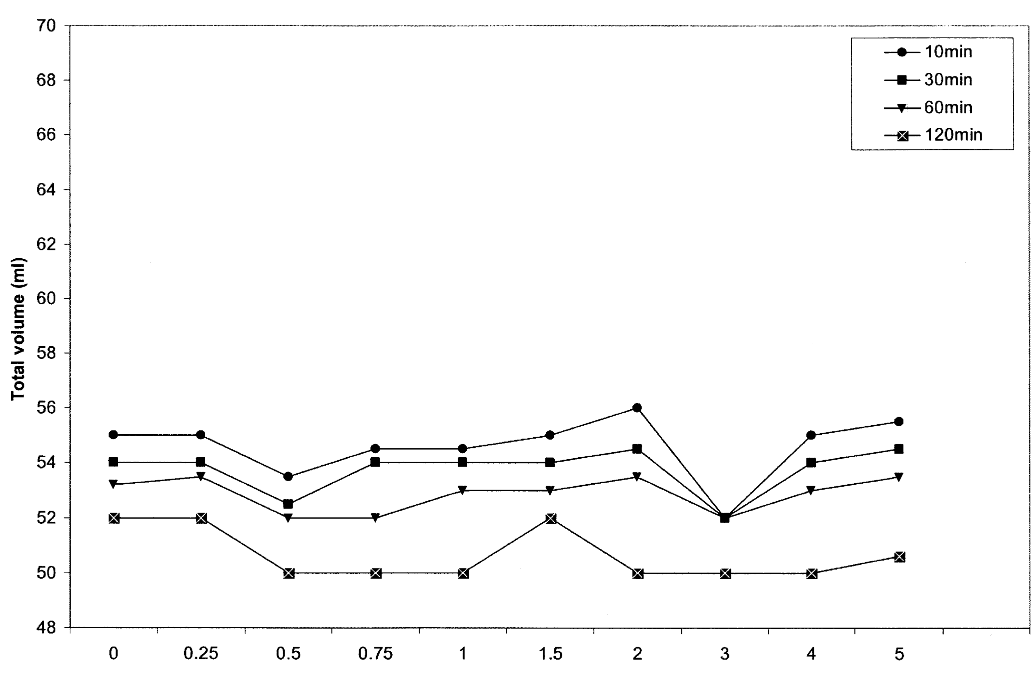

Fig. 3. Effect of NaCl and KCl at various concentrations on DCCF foaming stability.

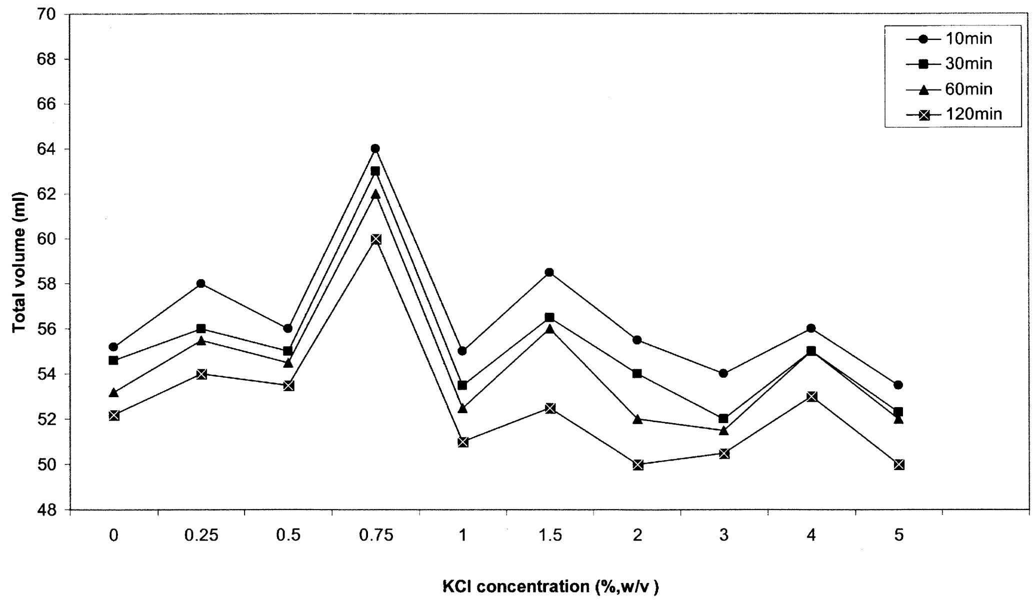

Fig. 4. Effect of KCl at various concentrations on DCCF foaming stability.

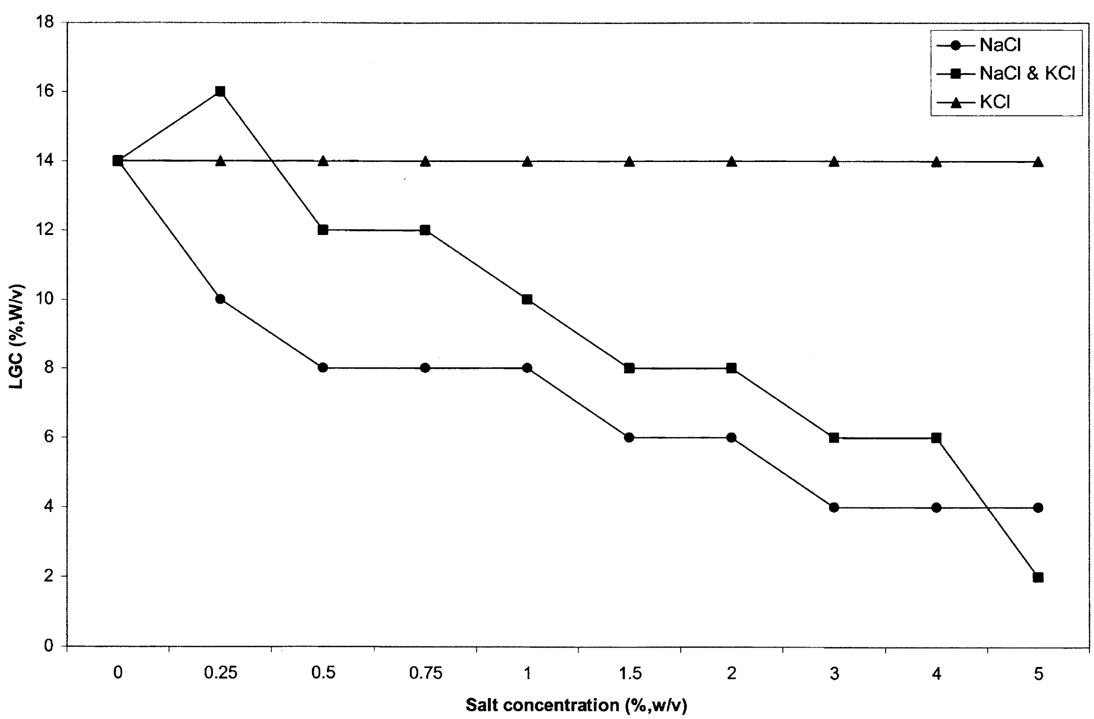

Fig. 5. Effect of salt on least gelation concentration (LGC) profile of DCCF.

reduced to 2% (w/v) in 5% salt mixture solution. However, complete replacement of NaCl with KCl had no effect on its LGC property. Oshodi and Ojokan (1997) reported an improvement in LGC of bovine plasma protein concentrate in NaCl and KCl up to 20% salt solution concentration, while Akintayo et al. (1999) reported an improvement in gelation capacity of pigeon pea (*Cajanus cajan*) protein concentrate only in 0.25% NaCl solution. The varied gelation properties of different legumes, as observed above, may be due to the different ratios of their constituent proteins, carbohydrates and lipids and interaction of such components (Padilla et al., 1996). Also gelation properties depend on the concentration and type of salts under consideration (Oshodi & Ojokan, 1997).

The improvement of *C.citrullus* LGC in NaCl and NaCl & KCl will enhance its use in new product development, where increased gel strength is desirable, i.e. comminuted sausage products (Oshodi & Ojokan, 1997).

4. Conclusion

For use of DCCF in new product formulation, where increased gelation strength and high protein solubility properties are desirable, KCl cannot effectively replace NaCl. However hypertensive patients are not at total disadvantage since a partial replacement of NaCl with KCl makes no significant difference. But a complete substitution of NaCl with KCl, at an appropriate concentration, offers the same or even better water absorption capacity and foaming capacity/stability with *C. citrullus* L.

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