



EFFECT OF SPROUTING USING SALINE WATER ON CHEMICAL COMPOSITION AND PROTEIN QUALITY AND FRACTIONATIONS OF EGYPTIAN CLOVER SPROUTS

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ABSTRACT

The effect of salt stress on growth proximate analysis, amino acid profile, protein quality and fractionations in 3 days etiolated clover sprout samples was investigated. Sterilized and non-sterilized clover seeds were sprouting using tap water 1000 ppm and 2000 ppm NaCl solution. The results showed reduction effect of clover sprout characters with higher NaCl concentration. Clover seed sprouting increased the crude protein content using tap water or saline water for sprouting as compared with dry seeds. However using non-sterilized clover seeds for sprouting recorded the higher values of protein (44.73%), lipids (6.21%) and energy (318.51 kcal/g) in etiolated sprouts, while using sterilized seeds recorded higher carbohydrate (21.28%), fiber (14.57%) and ash (4.46%). For amino acids, aspartic acid followed by glutamic acid were the most abundant, while Cysteine and methionine were the least in clover etiolated sprouts. Using saline water for clover seed sprouting caused increases in all amino acid compared with tap water except Methionine, aspartic acid and cysteine. For protein efficiency ratio (PER), essential amino acid index (EAAI%) and biological value (BV), from using saline water for sprouting had the higher values than sprouts from using tap water, but the values are less than dry seeds. On the other hand nutritional index (NI)

recorded the higher values in salt stress compared with both using tap water and dry clover seeds. For protein fraction in etiolated clover sprouts albumin was the major protein fraction extracted from NaCl 2000ppm sprout followed by prolamin from tap water sprouts, glutelin from NaCl 1000 ppm sprout and globulin from tap water sprout. The clover sprout protein isolated and its fractions could have excellent applications for future product development by virtue of their nutritional and functional properties.

INTRODUCTION

As Egypt population increases rapidly and it is consider one of the main constraints for limiting land, water and food resources, it's more important to define accurately the amount and quality of protein required to meet human nutritional needs. From nutritional point of view, clover or alfalfa sprouts are considered as a high quality for the health, due to its rich nutritional profile (>50 % protein) (Cairney 1997 and Abdallah 2008).

Clover (Berrsem) was first cultivated thousands of years ago in Egypt before pyramid building (Jones et al 1982 and Abdallah (2008) reported that seed germination and production of sprouts is an old habit that was adapted 3 days of sprouting to be the optimum period for clover growing in the dark to produce etiolated sprouts. Clover sprouts are the most common usually eaten raw in salads, therefore its recommends that, prior to sprouting, seeds be disinfected by washing in solution of 2% Calcium hypochlorite (CaCl₂O₂) (Lang et al 2000).

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In arid and semi-arid areas, salinity is the most serious a biotic stress that effects plant morphology and physiology through osmotic and ionic stress and changes biochemical response in plant (**Khan et al 2013**). Plants can resist osmotic stress by increasing the size of root system or reducing leaf area (**Guo et al 2002; Han and Wang, 2005**). Plants resist osmotic stress also by changing physiological and biochemical process such as antioxidant defense systems, solute accumulation, etc. (**Hasegawa et al 2000, Hsiao and Xu, 2000 and Ibrahim et al 2017**).

Proline quality evaluation aims to determine the capacity of food protein sources and diets to meet the essential amino acids needs. Compared to seeds, the sprouts have a higher quality of protein and more favorable amino acid composition (**Urbano et al 2005, Wang, et al 2005; Abdallah 2009**). The greatest increase during sprouting appears to be in glutamic and aspartic acids. (**Rozaan et al 2001; Abdallah et al 2009 and Ibrahim, 2017**). The salt stress caused increase level of amino acids proline, glutamic acid, aspartic acid and arginine in cereal **Simon-Sarkadi et al (2002)**. Moreover, during sprouting the water soluble protein content increased, but the salt soluble protein fraction decreased (**Wanasundara et al 1999**).

Albumins are water-soluble proteins, whereas globulins are salt (NaCl)-soluble proteins, and both were the major protein fractions, followed by storage proteins, glutelin (an organic solution – soluble proteins) in both wheat and barley seeds (**Xue Zhu et al 2006 and Kumar and Matta, 2011**).

During barley seed germination Albumins and globulins degraded faster. While glutelin and prolamin fractions were not seen degradation products (**Kumar and Matta, 2011**).

The Present investigation were under taken for determine the changes occurring in four protein fractions during sprouting of clover seeds using saline water and chemical composition investigate and protein quality of clover sprouts. The outcomes of this study would be important as a functional food ingredients.

MATERIALS AND METHODS

This study was carried out in Horticulture Department, Faculty of Agriculture, Ain Shams University, Cairo, and the Regional center for Food and Feed (RCFF), Agricultural Research center (ARC), Giza, Egypt. in the period (2016-2017).

Materials

Egyptian clover seeds (*Trifolium aelxandrium*) were obtained from the Crops Research Institute Agriculture Research Center, Giza Egypt. The seeds were cleaned from all impurities for sprouting, Calcium Hypochlorite 70%, Sodium chloride, absolute Ethanol and Sodium Hydroxide. Were obtained from Ahram Company, Cairo.

Methods

Seed sprouting

Sprouting of clover seeds was carried out in the glass jar as described by **Abdallah (2008)**. The clover seeds were sterilized in Calcium Hypochlorite 2% for 20 minutes before soaking in tap water or in NaCl 1000 and 2000 ppm solution for sprouting procedure. The other half of seeds were left unsterilized and soaking in bath tap water and NaCl solutions in the same sprouting procedure. Three days old Egyptian clover sprout from each Sprouting treatments and dry seeds (oven dried at 60°C for 72 hr.) were ground to pass through a 40 mesh sieve, the samples were stored until analysis at 5°C. Samples of harvested Three days old etiolated clover sprouts were taken from each treatment for measuring sprout characters (length of hypocotyl day and sprout and fresh weight of ten sprout and hypocotyl day).

Proximate analysis

Ash, total protein, lipids and crude fiber contents of samples were determined according to **AOAC (2012)**. Total carbohydrates were determined by subtracting. Energy value was calculated using the method [(9 x fat) + (4 x carbohydrate) + (4 x protein)] as described by **Chinma and Igyor (2007)**.

Amino acid Analysis

Amino acid determination was performed in Regional Center for Food and Feed, Agriculture Research Center, Giza, Egypt according to **AOAC (2012)** by using performic oxidation method (applicable to determination of amino acid (including methionine and cystine) in feeds. Performic acid oxidation is performed prior to hydrolysis to oxidize cystine and methionine to cysteic acid and methionine sulfone. Hydrolysis was carried out in closed conical flask for determining all amino acids other

than tryptophan. Sample equal to 10 mg of Protein was weighed in the conical flask and 5ml. of per formic acid was added. The flask was closed and placed in ice bath for 16 h. Sodium disulfite was added, 25 ml HCL 6N was added to the oxidized mixture. The flask was placed in an oven at 110^o for 24 h. The flask was then opened using Rotary evaporator to reduce the volume 5–10 MI under vacuum at 60 °c. Adjust the PH to 2.20 with sodium Hydroxide solution. Suitable volume of sodium citrate buffer (PH 2.20) was added to hydrolyzed sample. After all soluble material completely, dissolved; the sample is ready for analysis. The system used for the analysis was high performance Amino Acid analyzer (Biochrom 30).

Nutritional quality of clover sprouts

Sprouts samples Nutritional quality was determined using amino acid profiles and calculated essential amino acid index (EAAI) according to **Labuda et al (1982)** method according to the following equation:

$$EAAI = \frac{n8\sqrt{(Lys \times Iso \times val \times Thr \times leu \times Phe \times His \times meth)a}}{(lys \times iso \times val \times thr \times leu \times phe \times his \times meth)b} \times 100$$

Where:

n = number of essential amino acids, a,...= represent the concentration of essential amino acids [lysine, isoleucine, valine, threonine, leucine, phenylalanine, histidine and methionine] in test sample and , b,...= content of the same amino acids in standard protein (%) (**Tahany, Aly. et al 2018**).

Biological value was calculated according to **Oser (1959)** using the following equation:

$$BV = 1.09 \times \text{Essential amino acid index (EAAI)} - 11.7$$

The nutritional index percentage of sprout samples was determined using the following equation according to **Ijarorimi and Keshinro., (2013)**.

$$(NI)\text{Nutritional index (\%)} = \frac{EAAI \times \% \text{protein}}{100}$$

Protein Efficiency Ratio (C-PER) was estimated according to the regression equations developed by **Alsmeyer et al (1974)** as given below:

$$PER = -0.468 + 0.454(LEU) - 0.105(TYR)$$

Calculation of other protein quality parameters

From the results obtained for amino acids profile in seed sprouts. The total amino acids (TAA); total essential amino acids (TEAA); total neutral amino acids (TNAA); total acidic amino acids (TAAA); total aromatic amino acids (TArAA); and total basic amino acids (TBAA); were estimated. The ratio of (TEAA/TAA) and (% Cysteine/TSAA), also estimated.

Protein fractionation: using the procedure of **Osborne (1909)** with minor modifications was determined according to **Toukara et al (2013)**.

Statistical analysis

The data were statistically analyzed by analysis of variance using completely randomized design and least significant difference (L.S.D) at 0.05 levels according to the method described by **Snedecor and Cochran (1980)**.

RESULTS AND DISCUSSION

Effect of sprouting using saline water on sprout characters

Data in **Table (1)** showed no significant difference ($p \leq 0.05$) between sterilized and non-sterilized seed on 3days old clover etiolated sprout.

The length Sprout (6.12cm) and hypocotyl length (3.10cm) and heavier fresh weight (281.9 and 237.8 mg respectively) were observed when using tap water for sprouting as compared with using 1000 and 2000 ppm NaCl. The interaction effect between seed sterilization and NaCl concentrations showed the higher sprout length characters in sterilization with using tap water but in sprouts fresh weight the non-sterilized seeds with tap water recorded the heavier weight. The lowest sprout characters was recorded with increasing NaCl concentration (2000 ppm). Similar results on the reduction effect of sprout characters with higher NaCl concentration were reported by **Ghoulam and Fares (2001)**; **Ibrahim (2017)** and **Abd El-ghany (2017)**.

Table 1. Effect of NaCl concentration in sprouting solution clover etiolated sprout sterilized seeds characters (average of two experiments)

Sterilization (ste)	NaCl concentration	Hypocotyl length (cm)	Sprout length (cm)	10 Hypocotyl fresh weight (mg)	10 Sprout fresh weight (mg)
Sterilized seeds	Tap water	3.23 ^a	6.27 ^a	237.18 ^a	279.00 ^a
	1000	2.5 ^c	5.10 ^e	198.16 ^b	237.66 ^{bc}
	2000	2.47 ^c	5.23 ^{de}	199.93 ^b	235.69 ^c
	Mean	2.73 ^A	5.53 ^A	211.76 ^A	250.78 ^A
Non-sterilized seeds	Tap water	2.97 ^{ab}	5.97 ^{ab}	238.40 ^a	284.77 ^a
	1000	2.77 ^{bc}	5.61 ^{bc}	222.55 ^a	256.33 ^b
	2000	2.6 ^c	5.51 ^{cd}	194.03 ^b	230.95 ^c
	Mean	2.78 ^A	5.70 ^A	218.33 ^A	257.35 ^A
Average	Tap water	3.1 ^A	6.12 ^A	237.79 ^A	281.89 ^A
	1000	2.63 ^B	5.36 ^B	210.35 ^B	247.00 ^B
	2000	2.53 ^B	5.37 ^B	196.98 ^B	233.32 ^C
L.S.D (0.05)	Ste	NS	NS	NS	NS
	NaCl	0.23	0.27	13.77	13.65
	stexNaCl	0.33	0.38	19.47	19.30

Means in each column followed by the same letter are not significantly different at the 5% level ($p \leq 0.05$)
NS= non -significant.

Proximate analysis of sprout

The results of the proximate analysis of clover etiolated sprouts from sterilized and non-sterilized seeds and dry seeds are summarized in **Table (2)**. Non-sterilized seeds recorded the higher values of moisture content (9.48%), crude protein (44.73%), lipids (6.21%) and energy (318.51 kcal/g), while sterilized seeds recorded higher carbohydrate (21.28%), fiber (14.57%), ash (4.46%). Using saline water (1000 and 2000 ppm NaCl) for sprouting decreased sprout moisture percent and data was more pronounced using sterilized seeds. On the other hand, using tap water or saline water increased crude protein value as compared with dry seed, with more pronounced using tap water for sprouting which recorded more than 24% increment in protein compared with dry seeds. The highest values of crude protein in 3days old clover

etiolated sprouts, could be due to a decrease in dry matter through respiration of young sprouts, due to there were no nitrogen source added externally to the water and saline solution used for irrigation during sprouting. This crude protein % increase was therefore not a likely true increase (**Chavan and Kadam 1989, Abdallah 2008 and Dung et al 2010**). Carbohydrate values by difference recorded opposite results with protein and showed clear eased volute in sprouts compared with dry seeds speedily when using tap water for sprouting (**Table 2**). Lipid, fiber and ash value increased in clover sprouts than dry seeds. The fiber increased with increasing NaCl concentration, while ash decreased with increasing NaCl concentration. Clover etiolated sprouts relabeled lower energy value as compared with dry seeds. These observation may be clue to decreased in carbohydrate content.

Table 2. Proximate analysis (g /100 g),and Energy (Kcal/g) of 3 day old etiolated clover sprout

Sterilize (ste)	NaCl concentra	Moisture	Crude protein	Carbohy	Lipids	Fiber	Ash	Energy (Kcal/g)
Sterilized seed NaCl, ppm	Dry seed	9.58 ^b	38.57 ^f	33.12 ^a	4.28 ^g	10.59 ^g	3.85 ^f	325.34 ^a
	Tap water	9.23 ^{cd}	47.45 ^b	16.16 ^e	6.77 ^d	14.84 ^f	5.55 ^a	315.34 ^{cd}
	1000	8.93 ^f	47.57 ^b	17.37 ^d	6.26 ^e	15.88 ^b	3.99 ^e	316.09 ^c
	2000	8.98 ^{ef}	44.36 ^e	18.46 ^c	6.80 ^c	16.95 ^a	4.45 ^d	312.47 ^f
Mean		9.18 ^B	44.49 ^B	21.28 ^A	6.03 ^B	14.57 ^A	4.46 ^A	317.31 ^B
Non-Sterilized Seed NaCl, ppm	Dry seed	9.58 ^b	38.57 ^f	33.12 ^a	4.28 ^g	10.59 ^g	3.85 ^f	325.34 ^a
	Tap water	9.95 ^a	48.28 ^a	15.07 ^f	6.84 ^b	15.10 ^e	4.75 ^c	315.00 ^{de}
	1000	9.10 ^{de}	46.48 ^c	16.29 ^e	7.56 ^a	15.51 ^d	5.05 ^b	319.15 ^b
	2000	9.28 ^c	45.57 ^d	19.23 ^b	6.15 ^f	15.78 ^c	3.99 ^e	314.54 ^e
Mean		9.48 ^A	44.73 ^A	20.93 ^B	6.21 ^A	14.25 ^B	4.41 ^B	318.51 ^A
Average NaCl, ppm	Dry seed	9.58 ^B	38.57 ^F	33.12 ^A	4.28 ^G	10.59 ^G	3.85 ^F	325.34 ^A
	Tap water	9.59 ^A	47.87 ^A	15.62 ^B	6.81 ^B	14.97 ^C	5.15 ^A	315.17 ^C
	1000	9.02 ^C	47.03 ^B	16.83 ^C	6.91 ^A	15.70 ^B	4.52 ^B	317.62 ^B
	2000	9.13 ^B	44.96 ^C	18.85 ^B	6.48 ^C	16.37 ^A	4.22 ^C	313.50 ^D
LSD (0.05)	Ste	0.081	0.077	0.135	0.011	0.026	0.038	0.389
	NaCl	0.114	0.108	0.191	0.015	0.037	0.053	0.550
	Ste xNaCl	0.161	0.153	0.269	0.021	0.053	0.075	0.778

Means in each column followed by the same letter are not significantly different at the 5% level ($p \leq 0.05$).

Amino acid profile and quality of sprout

The amino acid profile of 3 days etiolated Egyptian clovers sprout samples are presented in Table (3). Data showed that sterilized clovers seeds before sprouting decreased four amino acids (Leucine, Glutamic acid, Arginine, and Glycine) and increased aspartic acid only as compared with non-sterilized seeds. Other amino acids not affected by seed sterilized (Table 3).

Concerning the effect of NaCl concentration effects data showed that sprouting in tap water and NaCl especially in low concentration (1000 ppm) decreased all essential amino acid content and non-essential amino acid compared with dry seeds, except high NaCl concentration (2000ppm) for Valine, Alanine, Serine and Tyrosine amino acids.. However, opposite results recorded for aspartic acid which recorded the lowest value in dry seeds and highest value in NaCl 2000 ppm.

The interaction between seed sterilization and NaCl concentration showed that non sterilized seeds sprouting using tap water recorded the lowest essential and non-essential amino acid value expect Methionine, Aspartic acid, and Cysteine amino acids which recorded the lowest values with using NaCl at higher concentration (2000 ppm) Leucine and using lower NaCl concentration (1000

ppm) for Cysteine and in dry seeds for Aspartic acid. On the other hand dry seed sample recorded the higher essential amino acids value except Valine and Phenylalanine which showed the high value with higher NaCl concentration (2000 ppm) interact with non-sterilized seeds. Also, dry seed sample recorded the higher non-essential amino acids value, except aspartic, alanine, serine and tyrosine which recorded the higher values with higher NaCl concentration (2000 ppm) interact with non-sterilized seeds, Also for Alanine, Serine and Tyrosine, but the higher values for aspartic acid was recorded with lower NaCl concentration (1000 ppm) interact with sterilized seeds (Table 3).

Using saline water at higher concentration (2000 ppm NaCl) for non-sterilized seed sprouting increased the content of Valine, Phenylalanine, Alanine, Serine and Tyrosine pile using tap water for sprouting decreased of almost all amino acids except Methionine, Aspartic acid and Cysteine.

Data in Table (3) also showed that aspartic acid was found to be the most abundant in sterilized or non-sterilized seed sprouts followed by glutamic acid. The higher aspartic and glutamic acid was recorded with higher NaCl concentration sprouts (13.77 and 10.82 g/100 g, respectively).

Opposite results was shown in dry seeds which recorded lower Aspartic acid (9.05 g/100 g) compared with Glutamic acid (14.47 g/100 g).

An observation in Aspartic acid /Glutamic acid showed that the level of Aspartic appeared to affect the glutamic (Table 3). The lowest Aspartic

acid / Glutamic acid was recorded in dry seeds (0.62) followed by sprouts using NaCl during sprouting (1.27), while, the highest Aspartic acid / Glutamic acid was recorded in clover sprouts using tap water for sprouting.

Table 3. Amino acids composition (g/100g protein) of 3 days etiolated Egyptian clover sprouts

Para met	EAA	Ile	Ther	Val	Phe	Lys	Leu	Meth	His	TEAA
Sterilized Seeds NaCl, ppm	Dry seed	3.26 ^a	2.57 ^a	3.91 ^b	3.94 ^a	5.53 ^a	5.75 ^a	0.98 ^a	2.56 ^a	28.53 ^a
	Tap water	2.87 ^c	2.19 ^d	3.65 ^d	3.71 ^b	3.77 ^e	4.85 ^{cd}	0.78 ^b	2.17 ^d	24.00 ^c
	1000	2.75 ^d	2.26 ^{cd}	3.68 ^{cd}	3.59 ^c	3.95 ^{cd}	4.61 ^e	0.91 ^{ab}	2.25 ^c	24.01 ^c
	2000	2.86 ^c	2.23 ^{cd}	3.77 ^c	3.63 ^c	3.88 ^{de}	4.78 ^d	0.81 ^b	2.13 ^{de}	24.11 ^c
Mean		2.93 ^A	2.31 ^A	3.75 ^A	3.71 ^A	4.28 ^A	5.00 ^B	0.87 ^A	2.27 ^A	28.53 ^A
Non Sterilized Seeds NaCl, ppm	Dry seed	3.26 ^a	2.57 ^a	3.91 ^b	3.94 ^a	5.53 ^a	5.75 ^a	0.98 ^a	2.56 ^a	28.53 ^a
	Tap water	2.59 ^e	2.03 ^e	3.43 ^e	3.42 ^d	3.42 ^f	4.47 ^f	0.89 ^{ab}	2.03 ^f	22.29 ^d
	1000	2.86 ^c	2.30 ^c	3.70 ^{cd}	3.65 ^{bc}	4.06 ^c	4.95 ^c	0.82 ^b	2.07 ^{ef}	24.55 ^c
	2000	3.14 ^b	2.43 ^b	4.15 ^a	3.97 ^a	4.36 ^b	5.29 ^b	0.83 ^b	2.46 ^b	26.77 ^b
Mean		2.96 ^A	2.33 ^A	3.79 ^A	3.74 ^A	4.34 ^A	5.12 ^A	0.88 ^A	2.28 ^A	25.53 ^A
Average NaCl, ppm	Dry seed	3.26 ^A	2.57 ^A	3.91 ^B	3.94 ^A	5.53 ^A	5.75 ^A	0.98 ^A	2.56 ^A	28.53 ^A
	Tap water	2.73 ^D	2.11 ^C	3.54 ^C	3.56 ^D	3.60 ^D	4.66 ^D	0.83 ^B	2.10 ^D	23.14 ^D
	1000	2.81 ^C	2.28 ^B	3.69 ^B	3.62 ^C	4.00 ^C	4.78 ^C	0.86 ^B	2.16 ^C	24.28 ^C
	2000	3.00 ^B	2.33 ^B	3.96 ^A	3.80 ^B	4.12 ^B	5.04 ^B	0.82 ^B	2.29 ^B	25.44 ^B
LSD(0.05)	Ste	NS	NS	NS	NS	NS	0.06	NS	NS	NS
	NaCl	0.07	0.06	0.07	0.05	0.1	0.08	0.1	0.04	0.47
	Ste X NaCl	0.1	0.08	0.1	0.07	0.15	0.12	0.15	0.06	0.67

Means in each column followed by the same letter are not significantly different at the 5% level ($p \leq 0.05$). Ile= Isoleucine, Ther= Therionine, Val= Valine, Phe= Phenylalanine, Lys= Lysine, Leu= Leucine, Meth= Methionine, His= Histidine, TEAA = Total Essential Amino Acids and NS= non-significant.

Cont. Table .3.

Parameter	NEAA	Asp	Ala	Glu	Ser	Arg	Pro	Gly	Cys	Tyr	NEAA	TAA
Sterilized Seeds NaCl, ppm	Dry seed	9.05 ^e	3.59 ^{ab}	14.47 ^a	3.47 ^b	7.92 ^a	3.66 ^a	3.58 ^a	1.63 ^a	3.19 ^{ab}	50.55 ^a	79.05 ^a
	Tap water	14.02 ^b	3.46 ^c	10.11 ^c	3.33 ^{bc}	4.99 ^{ef}	2.68 ^{bc}	2.87 ^c	1.41 ^b	3.01 ^c	45.26 ^c	69.27 ^c
	1000	14.43 ^a	3.45 ^c	9.53 ^{cd}	3.46 ^b	5.15 ^{de}	2.75 ^{bc}	2.80 ^c	0.45 ^d	3.07 ^{bc}	45.19 ^c	69.20 ^c
	2000	13.89 ^b	3.57 ^{ab}	9.88 ^c	3.18 ^c	5.23 ^d	2.61 ^c	2.86 ^c	0.57 ^d	3.04 ^c	45.24 ^c	69.35 ^c
Mean		12.84 ^A	3.52 ^A	10.99 ^B	3.36 ^A	5.82 ^B	2.92 ^A	3.03 ^B	1.01 ^A	3.08 ^A	46.57 ^A	71.73 ^A
Non Sterilized seeds NaCl, ppm	Dry seed	9.05 ^e	3.59 ^{ab}	14.47 ^a	3.47 ^b	7.92 ^a	3.66 ^a	3.58 ^a	1.63 ^a	3.19 ^{ab}	50.55 ^a	79.05 ^a
	Tap water	13.37 ^c	3.19 ^d	9.07 ^d	2.77 ^d	4.78 ^f	2.37 ^d	2.74 ^c	0.78 ^c	2.82 ^d	42.55 ^d	64.84 ^d
	1000	12.12 ^d	3.48 ^{bc}	11.24 ^b	3.48 ^b	5.51 ^c	2.81 ^b	3.49 ^a	0.56 ^d	2.99 ^c	45.60 ^c	70.15 ^c
	2000	13.66 ^{bc}	3.61 ^a	11.76 ^b	3.85 ^a	5.86 ^b	2.67 ^{bc}	3.14 ^b	0.96 ^c	3.22 ^a	48.35 ^b	75.13 ^b
Mean		12.05 ^B	3.47 ^A	11.63 ^A	3.39 ^A	6.02 ^A	2.88 ^A	3.24 ^A	0.98 ^A	3.05 ^A	46.77 ^A	72.31 ^A
Average NaCl, ppm	Dry seed	9.05 ^E	3.59 ^{AB}	14.47 ^A	3.47 ^B	7.92 ^A	3.66 ^A	3.58 ^A	1.63 ^A	3.19 ^{AB}	50.55 ^A	79.05 ^A
	Tap water	13.69 ^A	3.32 ^C	9.59 ^C	3.05 ^B	4.88 ^D	2.52 ^C	2.80 ^D	1.09 ^B	2.91 ^C	43.90 ^D	67.05 ^D
	1000	13.23 ^B	3.47 ^B	10.38 ^B	3.47 ^A	5.33 ^C	2.78 ^B	3.15 ^B	0.50 ^D	3.03 ^B	45.39 ^C	69.68 ^C
	2000	13.77 ^A	3.59 ^A	10.82 ^B	3.52 ^A	5.54 ^B	2.64 ^C	3.00 ^C	0.76 ^C	3.13 ^A	46.80 ^B	72.24 ^B
LSD (0.05)	Ste	0.18	NS	0.35	NS	0.11	NS	0.08	NS	NS	NS	NS
	NaCl	0.25	0.07	0.5	0.13	0.16	0.11	0.11	0.13	0.09	0.86	1.3
	SteX NaCl	0.36	0.11	0.71	0.19	0.22	0.16	0.16	0.19	0.13	1.22	1.84

Means in each column followed by the same letter are not significantly different at the 5% level ($p \leq 0.05$). Asp= aspartic acid, Ala= Alanine, Glu= glutamic acid, Ser= serine, Arg= arginine, Pro= proline, Gly= glycine, Cys= cysteine, Tyr= tyrosine, NEAA= non-essential amino, TAA= total amino acid and NS=non-significant.

The results of amino acid quality of clover seeds and etiolated sprouts are presented in **Table (4)**. Dry clover seeds and sprouts using higher saline solution concentration (2000 ppm NaCl) showed the highest percentage ratio of essential amino acid to the total amino acid (TEAA/TAA) about 36.1% and 35.2%, respectively. These values were above the 26% for ideal protein for children and 11% for adult (**FAO/WHO/UNU, 1985**). However dry seeds and higher NaCl concentration sprouts recorded lower TNEAA/TAA percentage (about 63.94 and 64.85%, respectively).

Total acidic amino acid (TAAA) ranged in clover sprouts from 23.27 to 24.59 (**Table 4**) which was almost close to the total essential amino acid (TEAA from 23.14 to 25.44 as in **Table 3**). Whilst, the percentage range in total basic amino acid (TBAA) from 8.5 to 13.4 that made them the third largest amino acid group among etiolated clover sprouts samples.

Sterilized and non-sterilized seeds recorded cysteine /Total Sulfur amino acid TSAA percentage more than 50% (53.72% and 52.68%, respectively). Moreover, dry clover seeds and etiolated sprouts contain more cysteine than methionine. While opposite results was obtained using NaCl solution for sprouting. For examples cysteine /TSAA percentage was 62.43% in dry seeds and 56.77% in etiolated clover sprouts germinated in tap water followed by 48.1% and 36.76% in sprouts germinated in saline water (2000 ppm and 1000 ppm NaCl, respectively). However, NaCl at 2000 ppm interact with non-sterilized seeds contain more cysteine than methionine. (Cysteine /TSAA percentage was 53.63%).

Adeyeye (2004) reported that many vegetables protein contains substantially more cysteine than methionine. Similar data was obtained by **Ahmed et al (2018)**. In contrast, animal proteins are low in cysteine (**Adeyeye and Ayejuyo, 2007**). However, cysteine has positive effects on mineral absorption particularly zinc (**Sandstrom et al 1989 and Mendoza, 2002**).

The total aromatic amino acid (TAA) of etiolated clover sprout were ranged from 6.5 g/100g using tap water for sprouting to 6.93 g/100 g using saline water (2000 ppm NaCl) for sprouting as compared with dry seeds (7.13 g/100g).

The assay methods for estimating protein quality employ casein as the reference protein. However the high quality protein used as reference should be determined at the same time and under the same conditions as the assay procedure being

used **FAO/WHO,(1991)** therefore using standard protein (casein) in these research for estimating protein and amino acid quality is recommended even it is not identical to reference protein as reported by **Tahany, Aly. (2018)**.

The most widely used method for measured of protein quality is protein efficiency ratio (PER) test, which is the weight gain per weight of protein eaten. The values of PER of etiolated clover sprouts sample was between 2.28 using tap water for sprouting to 2.42 using NaCl 2000 ppm solution for sprouting compared to 2.74 in dry clover seed sample (**Table 4**). The higher PER value in clovers sprouts was (2.53) recorded when using non sterilized seeds sprouting using NaCl 2000 ppm followed by NaCl 1000 ppm (2.4).

These increments in PER value can explain by the increment in leucine and tyrosine amino acid composition (**Table 3**). The PER value in clover sprouts using saline water (2.53) was equal for that recorded in reference (2.5) (**Oyarekua and Eleyinmi, 2004**), but was higher than PER recorded in cowpea (1.21) and pigeon pea (1.82) (**Salunkhe and Kadom, 1989**) and green radish sprout (1.69) (**Tahany, Aly. et al 2018**).

Clover etiolated sprouts in both tap and saline water decreased essential amino acid index (EAAI) to (56.45-62.21%) compared with dry seeds (68.25%), similar to PER values. Also biological value (BV) showed similar decrement (48.77-52.86%) than dry seeds (61.63%). However, clover sprouts using saline water increased EAAI and BV than using tap water. These results showed that consumption of etiolated clover sprouts alone as food are in adequate and in agreement with good nutritional quality (EAAI and BV less than 10 to 100%) for food, base-protein (**Oser, 1959**). similar result was obtained with wheat sprout flour samples (**Ibrahim, 2017**) and green radish sprouts (**Tahany, Aly. 2018**).

Regarding nutritional index (NI), data in **Table (4)** showed that etiolated clover sprouts in both tap and saline water increased nutritional index to (26.98-28.31) compared with dry clover seed sample (26.32). Using non sterilized clover seed for sprouting using NaCl 2000 ppm solution for sprouting recorded the higher nutritional index (29.86), while using NaCl concentration for sprouting sterilized clover seeds recorded the lower nutritional index value (26.11).

4- Effect of protein profiles

Changing levels of protein fractions were analyzed in etiolated sprouting clover at 3 days sprout stage. Data in **Table (5)** should that sterilized clover seed decreased of prolamins and increased of glutelins while that of albumins and globulin fraction registered non-significant effects during sprouting. However, using saline water (NaCl 1000 or 2000 ppm) for seed sprouting decreasing globulins and prolamins fractions while increased albumins and glutelin fractions in 3 days old etiolated sprouts as compared with using tap water for seed sprouting. The highest albumin and glutelin level was recorded using non sterilized sprouting with

2000ppm NaCl in traction for albumin and with 1000ppm NaCl for glutelin, while the lower globuling level was obtained when non -sterilized seed sprouting with 2000 ppm NaCl interaction but lower prolamin level was recorded when sterilized seed sprouting with 2000ppm NaCl.

Finally, Albumin was the major clover sprout protein fraction extracted from NaCl 2000 ppm sprout followed by prolamin from tap water sprouts, glutelin from NaCl 1000 ppm sprout and globulin from tap water sprouts.

The clover sprout protein isolated and its fractions could have excellent applications for future product development by virtue of their nutritional and functional properties.

Table 5. Effect of sterilized and non-sterilized clover seed sprouting using saline water (NaCl 1000 and 2000 ppm) on the protein fractionation of 3 days etiolated clover sprouts characters (mg /100mg sample)

Sterilization (ste)	Parameter	Albumin	Globulin	Glutelin	Prolamin
Sterilization NaCl, ppm	Tap water	46.83 ^e	7.50 ^a	14.47 ^d	31.20 ^a
	1000	52.97 ^c	5.73 ^b	18.83 ^b	22.47 ^e
	2000	54.40 ^b	4.80 ^c	19.30 ^b	21.50 ^f
Mean		51.40 ^A	6.01 ^A	17.53 ^A	25.05 ^B
Non sterilization NaCl, ppm	Tap water	48.73 ^d	7.83 ^a	15.23 ^c	28.20 ^b
	1000	46.20 ^e	6.30 ^b	22.20 ^a	25.30 ^c
	2000	58.10 ^a	3.73 ^e	14.17 ^d	24.00 ^d
Mean		51.01 ^A	5.95 ^A	17.20 ^B	25.83 ^A
Average NaCl, ppm	Tap water	47.78 ^C	7.67 ^A	14.85 ^C	29.70 ^A
	1000	49.58 ^B	6.02 ^B	20.52 ^A	23.88 ^B
	2000	56.25 ^A	4.27 ^D	16.73 ^B	22.75 ^C
LSD 0.05	Ste	NS	NS	0.29	0.30
	NaCl	0.66	0.43	0.36	0.37
	Ste×NaCl	0.93	0.61	0.51	0.53

Means in each column followed by the same letter are not significantly different at the 5% level (p≤0.05). NS=non -significant.

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