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EFFECT OF SOAKING AND SPROUTING USING SALINE WATER ON CHEMICAL COMPOSITION OF WHEAT GRAINS

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ABSTRACT

In the current research, wheat grains were used to study the effect of grain soaking and sprouting using tap water and saline water (NaCl solution) on sprout growth, proximate analysis, minerals content, anti-nutritional and antioxidant compounds of sterilized grains (soaked for 0.33h) and soaked grains for imbibition (12h) and sprouted grain for 24h old. Results revealed that the longest radical of 24h old wheat sprout was observed at 2000 ppm NaCl, and shortest was observed at 4000 ppm NaCl. Soaked wheat grains (12h) for imbibition recorded the highest moisture content (10.2 to 10.9%) while soaked for 20 min (0.33h) in calcium hypochlorite for sterilization recorded medium content (8.8 to 9.9%) and the lowest one recorded in 24h old wheat sprouts (6.9 to 7.2%). The low moisture content the high total carbohydrate, total fats and energy and vice versa. Soaked grains for sterilization period (0.33 h) and imbibition (12h) increased zinc (Zn), manganese (Mn) and calcium (Ca) while non-sterilized only potassium (K). Tap water increased sprout magnesium (Mg), and manganese (Mn) content while saline water increased sprout magnesium (Mg), and calcium (Ca) content. Grain sprouting was effective in reducing phytic acid, oxalate and alkaloids anti-nutrient in wheat sprouts especially when using sterilized grains. Soaking non sterilized grains for imbibition (12h) in saline water contained higher total phenol, flavonoids and total antioxidant. Etiolated wheat sprouts contained lower total

flavonoids and antioxidant compared with soaked grains in saline water.

Key wards: Wheat grains, Soaking, Sprouts, Saline water, proximate analysis, Minerals, Antinutrient, Antioxidant.

INTRODUCTION

Wheat is one of the first domesticated species to man and is also the first agricultural product used in food processing showing fundamental role in the human food base (Silva et al 2014). The wheat grain has important role in the economic and nutritional aspects of food because their flour is widely used in food industry for the production of flour especially in bread and pasta (Camargo et al 2004). Sprouting grains for human consumption has been used for centuries in Egypt and Asian counties to improve food value (Resh, 2001 and Abdallah, 2008). Therefore, the trend is to produce specially breads and backed goods from whole grain flour and seed sprouts known as functional foods (Dewettinek et al 2008, Jideani and Onwubali, 2009 and Abd allah and Abo El-Naga, 2013). Sprouting is the practice of soaking and leaving seeds until they germinate and begin to sprout. This practice is reported to be associated with improvements in the nutritive value of seeds (Zanabia et al 2006, Abdallah, 2008 and Kumar et al 2010). At the same time there are indications that germination is effective in reducing phytic acid (Kalapadevi and Mohan, 2013 and Ibrahim 2017), and other anti-nutrition of factors (AbdElAzim et al 2018). Imbibing grains under warm, moist conditions is the only means of determining the germ inability of wheat grains. Water entered the embryo and scutellum during the very early stages of imbibition through the micro pyle and by 2h of imbibition, embryo structures such as the coleoptile and radicle were clearly distinguished. Although water accumulated between the inner (seed coat) and outer (pericarp) layers of the coat surrounding the grain, there was no evidence for movement of water directly across the coat and into the underlying starchy endosperm (Rathjen et al 2009).

Salinity is one of the most serious a biotic stress that affects crop production in the arid and similar zone of the world. Seed germination and seedling growth are known to be more sensitive to salt stress compared with later development stages (Ashraf 1994 and Yildirim et al 2002). Salt stress negatively affects plant morphology and physiology through osmotic and ionic stress changes biochemical responses in plant (Khan et al 2013). On The other hand, salt stress stimulates the activity of antioxidant system (Rady. 2011 and Semida and Raoly, 2014).

Germination brought about significant increases in the micronutrient, phytonutrient content of all selected seeds, thus proving that there is marked increase in the nutritive value of the seeds on sprouting. This ultimately signifies that sprouts should be incorporated to improve agricultural productivity and easily to use by low income families (Wagner et al 2013).

The aim of the present study was to investigate the effect of soaking and sprouting using tap water and NaCl solution for soaked grains and one day sprout characters, proximate analysis, energy, minerals, antioxidants and anti-nutritional compounds of wheat grains

MATERIALS AND METHODS

This study was carried out in Horticulture Department, Faculty of Agriculture, Ain Shams University and the Regional Center for Food and Feed (RCFF), Agriculture Research center (ARC).

Materials

1- Wheat grains and NaCl

Dry wheat grains (*Triticumae stivum* L.) Cultivar Giza 168 was obtained from Agriculture Research Center, Giza. NaCl was obtained from El-Gomhoria chemical company, Cairo Egypt

2 - Grains sprouting.

Sprouting of cleaned sterilized by soaking for 20 min in calcium hypochlorite and non-sterilized whole wheat grain was dame in glass jar method for imbibition soaking (12 h) and others for sprouting as reported by Abdallah, (2008) using tap water and NaCl at 1000, 2000, 3000, 4000 ppm solution for grain soaking and sprouting, wheat sprout sharvested one day from grain soaking. Grains, soaked grains and harvested sprouts dried using air draft oven at 55+2°C for 48 hr. then grounded into powder for chemical analysis. Samples of sprouts were also collected for measuring sprout characters (radical length (cm), 100 sprouts fresh and dry weight (g), weight losses during sprout (%) and addition to measure imbibed Water ml/100g of seeds

3 - Chemical analysis

Moisture, total protein, lipids, crude fiber and ash contents of the samples were determined according to AOAC (2012). Total carbohydrate determined by subtracting. The energy value was calculated using the at water factor method [(9 x fat) + (4 x carbohydrate) + (4 x protein)] as described by Osborne and Voogl, (1978), Eneche (1991), Chinma, Igyor (2007) and Nwabueze (2007). Potassium (K), magnesium (Mg), iron (Fe), zinc (Zn), calcium (Ca) and manganese (Mn) were analysed by atomic absorption spectrophotometer 3300 perken Elmer, while calcium (Ca) was analyzed by ICP optima 2000 DV perken Elmer. According to the method described in the AOAC (2012). Concerning anti-nutrient analysis, total oxalate was determined through titration methods according to Day and Underwood (1986), phytic acid was determined based on precipitation of phytate according to the procedure of Wheeler and Ferr et al (1971) nitrate calibration curve. Total tannins were determined by spectrophotometric method as described by Makkar et al (1993) and alkaloids determined by procedure proposed by Harbone (1973) and further explained by Onwuka (2006). Saponin content of the samples was determined by double solvent extraction gravimetric method (Harbone 1973 and Obadoni and Ochuko 2001). The total antioxidant capacity of the samples was evaluated by the method of Prieto et al (1999). The Folin Ciocalteu method (Singleton, et al 1999) was used to determine total phenolic content. The total flavonoid content was determined using aluminium chloride colorimetric

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method as adapted by Arvouet Grand et al (1994).

4- Statistical analysis

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

RESULT AND DISCUSSION

1- Effect of NaCl concentrations in sprouting solution on grains imbibed water (12h) and one day old wheat grains etiolated sprout characters.

Data in **Table (1)** showed no statistical significant difference between sterilized and non sterilized grains in wheat sprout radical length, 100 sprout fresh and dry weights and dry weight losses percentage during 24h sprouting. But sterilization decreased grain imbibed water. Moreover no statistical difference between tap water and NaCl concentrations (1000, 2000, 3000 and 4000 ppm) in wheat sprouts fresh and dry weight and percentage of dry weight losses during sprouting. Concerning sprout radical length, the longest radical was observed at 2000 ppm NaCl with no different with the following observed using tap water, while the shortest radical length was observed at 4000 ppm NaCl followed by 3000 ppm . The higher NaCl concentration (4000 ppm) increased grain imbibed water compared with control. The interaction between sterilization and NaCl concentration recorded the tallest radical length in 2000 ppm NaCl followed by tap water with or without grain sterilization. The higher imbibed water was recorded with all NaCl concentration interacts with nonsterilized seeds. Therefore 2000 ppm NaCl was selected for the following study. Similar results on decreasing sprout length with increasing NaCl concentration were reported by Ibrahim (2017), Abd El- Azim et al (2018) and Basma Soliman et al (2018).

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 Table 1. Effect of NaCl concentrations in sprouting solution on grains imbibed water 12h and one day old wheat grains etiolated sprout characters

Sterilization (ST)	Na CI Concentration PPM	Radical length (cm)	100 Sprout fresh Weight (g)	100 Sprout dry weight (g)	weight Losses during sprout (%)	Imbibed water ml / 100g seeds
	Tap Water	0.244 a	7.286 a	4.53 a	6.54 a	61.95 d
v	1000	0.175 bcd	7.008 a	4.412 a	6.40 a	66.68 cd
Sterilized seeds	2000	0.250 a	6.939 a	4.342 a	5.19 a	72.44 bc
teri	3000	0.149 bc	6.817 a	4.287 a	5.01 a	65.70 cd
Ó	4000	0.115 d	6.855 a	4.361 a	6.31 a	75.43 abc
	Mean	0.187 A	6.981 A	4.386 A	5.89 A	68.44 B
σ	Tap Water	0.238 ab	7.183 a	4.389 a	4.94 a	78.64 ab
Non-Sterilized seeds	1000	0.189 abc	6.947 a	4.337 a	4.94 a	79.85 a
-Sterili seeds	2000	0.252 a	6.988 a	4.475 a	5.46 a	73.84 abc
set -St	3000	0.149 cd	7.247 a	4.394 a	5.92 a	72.70 abc
lon	4000	0.143 cd	7.102 a	4.564 a	6.68 a	82.98 a
2	Mean	0.194 A	7.093 A	4.432 A	5.59 A	77.60 A
	Tap Water	0.241 A	7.235 A	4.459 A	5.74 A	70.30 B
ge	1000	0.182 B	6.977 A	4.374 A	5.67 A	73.26 AB
Average	2000	0.251 A	6.963 A	4. 408 A	5.33 A	73.14 AB
Av	3000	0.149 BC	7.032 A	4.341 A	5.46 A	69.20 B
	4000	0.129 C	6.979 A	4.462 A	6.49 A	79.21 A
LSD 0.05	(ST)	NS	NS	NS ⁽¹⁾	NS	4.629
	NaCl	0.047	NS	NS	NS	7.32
	ST× Na Cl	0.066	NS	NS	NS	10.352

Means in each column followed by the same letter are not significantly different at the p<0.05

2- Proximate analysis and energy content of wheat grain soaked for 0.33h (sterilized period), soaked 12 h (imbibition period) and 24h sprouts.

Soaked wheat grains 12h for imbibitions recorded the higher moisture content in dry samples (10.2 to 10.9%) with and without grain sterilization and saline water (NaCl 2000 ppm) while soaked grains for 0.33h in calcium hypochlorite 2% for grain sterilization recorded medium moisture content (8.8 to 9.9 %). But moisture content of wheat sprouts 24 h old was the lowest with and without sterilization and saline water (6.9 to 7.2 %) as shown in **Table (2)**. The lower moisture content in wheat sprouts showed increase in total carbohydrates (74.55 to 74.89%), and total fats (2.01 to 2.07%) compared with other treatment. On the

other hand the higher moisture content in soaked grains for imbibitions 12 h showed decreased in carbohydrate (71.41 to 72.25%), fat (1.99 to 2.0%) and fiber (1.78 to 1.98%) compared with other treatments. Concerning protein and ash data showed close content between treatments for both. (Table 2). Regarding energy, the higher energy value (367.9 to 368.9) recorded in the lowest moisture content and higher carbohydrates and fat. Similar results were obtained by Abd El- Azim et al (2018) and Basma Soliman et al (2018). The higher energy value can discuss by increasing carbohydrates and fats with no clear changes in protein.

Since the energy value was calculated using the at water factor method (9 x fat) + (4 x carbohy-drate) + (4 x protein).

Table 2. The proximate analysis g/100gdw and energy content of wheat grain soaked for 0.33h (sterilized
period), soaked 12h (imbibition period) and 24h sprouts.

Treatment	Moisture	Carbohydrate	Protein	Fat	Fiber	Ash	Energy (Kcal/g)
sterilized grains -TW ⁽¹⁾ - ST ⁽²⁾ (0.33hr)	8.8	73.44	12.4	1.92	1.94	1.5	360.6
non-sterilized grains -TW - NST ⁽³⁾ (0.33hr)	9.9	71.45	13.3	1.95	2.00	1.4	356.6
soaked grains (12hr) -TW- ST	10.9	71.73	12.2	1.99	1.78	1.4	353.6
soaked grains (12hr) - TW – NST	10.2	72.25	12.3	2.00	1.85	1.4	356.7
soaked grains (12hr) -SW ⁽⁴⁾ - ST	10.7	71.41	12.7	2.00	1.79	1.4	354.4
soaked grains (12hr) -SW – NST	10.5	72.13	12.1	1.99	1.98	1.3	354.8
sprout- TW – ST	7.0	74.55	13.1	2.02	1.93	1.4	368.9
sprout- TW – NST	7.2	74.55	12.8	2.05	2.00	1.4	367.9
sprout SW – ST	6.9	74.89	12.6	2.01	2.10	1.5	368.1
sprout SW –NST	7.1	74.82	12.5	2.07	2.11	1.5	367.9

(1)TW=tap water (2) ST=sterilized seeds (3) NST=non-sterilized seeds (4) SW=saline water (NaCl 2000 ppm)

3- Minerals content of wheat grain soaked for 0.33h (sterilized period), soaked 12h (imbibition period) and 24 h sprouts.

Concerning effects of sprouting using saline water with sterilized and non-sterilized grains on mineral contents, data in **Table (3)** showed that sterilized dry grains for 0.33h in calcium hypochlorite 2% increased Zn, Mn and Ca while non-sterilized dry grains increased only K compared with other minerals. Moreover, sterilized grains soaked for 12h in tap water for imbibition increased grains Fe, Zn, Mn and Ca contents white non-sterilized grains soaked for 12h in tap water increased grains K content only compared with other minerals, also Fe content was increased in sterilized grains soaked for 12h in saline water. On the

other hand wheat grains sprout contain higher Mg and Mn using sterilized and non-sterilized grains sprouting in tap water, but using saline water decreased sprout Mg content and increased Ca content compared with other sprout.

4- Anti-nutrient compounds of wheat grain soaked for 0.33h (sterilized period), soaked 12h (imbibition period) and 24 h sprouts

The anti-nutrient compositions are presented in **Table (4).** Tannins were increased in non-sterilized dry wheat grains soaked for 20 minutes 0.33h and also increased in grains sprouts specialty when using tap water for sterilized and non-sterilized grains sprouting.

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	K	Mg	Fe	Zn	Mn	Ca
Minerals						
sterilized grains -TW ⁽¹⁾ - ST ⁽²⁾ (0.33hr)	700	48.92	0.5904	0.3452	0.1303	532
non-sterilized grains -TW - NST ⁽³⁾ (0.33hr)	1500	67.98	0.6160	0.0713	0.0293	405
soaked grains (12hr) -TW- ST	500	57.76	0.8929	0.348	0.1326	516
soaked grains (12hr) - TW - NST	1000	57.48	0.253	0.2220	0.0094	413
soaked grains (12hr) -SW ⁽⁴⁾ - ST	400	71.30	0.7555	0.084	0.0998	414
soaked grains (12hr) -SW - NST	500	64.04	0.5020	0.2173	0.1357	289
sprout- TW - ST	500	73.04	0.7387	0.1743	0.1813	386
sprout- TW - NST	400	72.94	0.1058	0.249	0.1278	376
Sprout SW - ST	500	53.58	0.7536	0.1013	0.0383	489
sprout SW - NST	500	52.58	0.6317	0.1159	0.0563	491

 Table 3. Minerals content (ppm) of wheat grain soaked for 0.33h (sterilized period), soaked 12h (imbibition period) and 24 h sprouts.

(1)TW=tap water (2) ST=sterilized seeds (3) NST=non-sterilized seeds (4) SW=saline water (NaCl 2000 ppm)

Table 4. Anti-nutrient compounds of wheat grain soaked for 0.33 h (sterilized period), soaked 12h (imbibition period) and 24 h sprouts.

Anti-nutritional compounds	Tannins %	PhyticAcid %	Oxalate %	Alkaloids %	Saponins %
sterilized grains -TW ⁽¹⁾ - ST ⁽²⁾ (0.33hr)	0.09	0.57	0.05	4.72	0.53
non-sterilized grains -TW - NST ⁽³⁾ (0.33hr)	0.16	0.48	0.076	8.59	1.56
soaked grains (12hr) -TW- ST	0.15	0.67	1.20	4.77	2.69
soaked grains (12hr) - TW - N ST	0.14	0.62	1.12	3.20	1.70
soaked grains (12hr) -SW ⁽⁴⁾ - ST	0.16	0.68	1.20	4.35	2.34
soaked grains (12hr) -SW - NST	0.14	0.69	1.10	3.20	1.90
sprout- TW – ST	0.17	0.58	0.95	1.95	0.67
sprout- TW – N ST	0.16	0.60	1.20	4.60	2.40
Sprout SW – ST	0.15	0.58	1.00	2.52	1.48
Sprout SW –N ST	0.14	0.59	1.10	3.18	1.93

(1)= tap water (2) ST=sterilized seeds (3) NST=non-sterilized seeds (4) SW=saline water (NaCl 2000 ppm)

The phytic acid and oxalate percentage were increased in soaking grains for imbibition's 12h while decreased in all sprouts treatments and in dry grains soaked for 0.33h during sterilization. However, **Kalpanadevi and Mohan (2013)** reported that germination is effective in reducing phytic acid. Alkaloids showed highest content (8.59%) in non-sterilized dry grains soaked for 0.33h in tap water, followed by sterilized dry grains, sterilized soaked grains 12h and non-sterilized grains sprouts using tap or saline water. Concerning saponins data showed elevation of saponin percentage in non-sterilized dry grains soaked for 0.33 h in tap water, and sterilized grain soaking 12h for imbibition, and non-sterilized grain sprout, in both tap and saline water the saponin used as precursor for the synthesis of steroid hormones. Also saponine especially diosgnin also exhibited anticancer, anti-diabetes, anti-microbial properties and anti-aging activities (Tada et al 2009, Yan et al 2009 and Chaudhary et al 2018). However scientific studies have established that germination improve the nutritional quality of food products by reducing or eliminating the anti-nutrient composition of food products (Mbithi– Mwikya et al 2001, Ibrahim 2017, and Abd El– Azim et al 2018).

5- Antioxidant compound of wheat grain soaked for 0.33h (sterilized period), soaked 12h (imbibition period) and 24 h sprouts.

Table (5) showed the effect of wheat sterilized grains for 0.33h soaked grains for 12h and etiolated sprout 24h old on total phenols, total flavonoids and total antioxidant content (ppm). Non sterilized grain soaked for 12h in saline water contained higher total phenols (2425 ppm), flavonoids (314 ppm) and total antioxidant (6337 ppm), than other treatments . However saline water increased total antioxidant in soaked grains for 12h. In contrast grains etiolated sprout in saline water for 24h contained lower total flavonoids, and antioxidant compared with soaked grains. The high increases in total phenol and flavonoids in 12h soaked grains may be due to that soaking processes synthesized these compounds with vitamin C as good antioxidant agents against salinity.

Table 5. Antioxidant compound (ppm) of wheatgrain soaked for 0.33h (sterilized period), soaked12h (imbibition period) and 24h sprouts.

	-	T ()	T
Antioxidant	Total	Total	Total
Compounds	Phenols	Flavonoids	Antioxidant
	ppm	ppm	Ppm
sterilized grains-TW ⁽¹⁾ - $ST^{(2)}$	1304.5	250.6	4813
(0.33hr)			
non-sterilized grains-TW-	844	250.6	3728
NST ⁽³⁾ (0.33hr)			
soaked grains (12hr)-TW- ST	1105	240.6	4079
soaked grains (12hr)- TW-	2156	240.6	5510
NST			
soaked grains (12hr)- $\mathrm{SW}^{(4)}\text{-}$	1716	250.4	5996
ST			
soaked grains (12hr)- SW -	2425	314.2	6337
NST			
sprout- TW – ST	2171	84.9	4313
sprout- TW – N ST	1943	112.1	4484
sprout SW – ST	1151	71.6	3830
sprout SW –N ST	1242	82.6	4014

TW= tap water (2) ST=sterilized seeds (3) NST=non-sterilized seeds (4) SW=saline water (NaCl 2000 ppm)

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تاثير النقع والتنبيت باستخدام الماء المملح على المكونات الكيميائيه لحبوب القمح

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تم في هذا البحث استخدام حبوب القمح لدراسه تاثير نقع الحبوب والتنبيت باستخدام الماء المملح بكلوريد الصوديوم مقارنه بماء الصنبور العادى على نمو النبت وتحليل المكونات والعناصر الغذائيه ومضادات التغذيه ومضادات الاكسده للحبوب المعقمة التي تم تعقيمها بالنقع لمده 20 دقيقه والحبوب المنقوعه 12 ساعه لاجراء عمليه التشرب بالمياه وكذلك نبت الحبوب بعمر 24 ساعه. واجريت هذه الدراسه في معمل الزراعه العضويه والخضروات النابته بقسم البساتين – كليه الزراعه – جامعه عين شمس و تم تقدير التحليلات الكيميائيه بالمركز الاقليمي للاغذيه والاعلاف- مركز البحوث الزراعيه. واظهرت نتائج البحث ان تركيز 2000 جزء في المليون من كلوريد الصوديوم ادى الى الحصول على اطول جذير في نبت القمح بعمر 24 ساعه، بينما كان الاقصر طولا للجذير في تركيز 4000 جزء في المليون من كلوريد الصوديوم وسجلت عمليه نقع الحبوب لمده 12 ساعه بغرض التشرب اعلى محتوى من الرطوبه وصلت الي 10.2-10.2%، بينما اظهرت الحبوب المنقوعه لمده 20 دقيقه للتعقيم في هيبوكلوريد الكالسيوم محتوى متوسط من الرطوبة 6.9 - 7.2 %، وإظهرت النتائج

تحکيم: ا د إمام عبدالمبدي

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أنه كلما انخفضت رطوبه العينات كلما زاد محتواها من الكربوهيدرات والدهون والطاقه و العكس صحيح، هذا واظهرت معامله نقع البذور للتعقيم لمده 20 دقيقه او النقع للتشرب لمده 12 ساعه زياده في محتوى عينات تلك الحبوب من عناصر الزنك والمنجنيز والكالسيوم، بينما ازداد البوتاسيوم فقط في عينات النقع لمده 20 دقيقه بدون تعقيم وبالنسبه لنبت القمح بعمر 24 ساعه قد ازداد محتواه من الماغنسيوم والمنجنيز عند التنبيت بإستخدام ماء الصنبور، بينما زاد الماغنسيوم والكالسيوم عند التنبيت باستخدام الماء المملح. كما ادت عمليه التنبيت الكفاءه في تقليل محتوى النبت من المضادات التغذويه مثل حمض الفايتك والاوكسالات والقلويدات خاصه عند استخدام الحبوب المعقمه بهيبوكلوريت الكالسيوم هذا وادى نقع حبوب القمح غير المعقمه لمده 12 ساعه بغرض التشرب في الماء المملح الي زياده محتواها من الفينولات والفلافنويدات ومضادات الاكسده وانخفض محتوى نبت الحبوب المنقوعه في الماء المملح من الفلافونيدات ومضادات الأكسدة.

الكلمات الداله: حبوب القمح، النقع، النبت، الماء المالح، تحليل المكونات، العناصر الغذائيه، مضادات تغذوية، مضادات الاكسده