Zagazig J. Agric. Res., Vol. 40 No. (3) 2013 559-570

Zagazig Journal of Agricultural Research

www.zu.edu.eg/agr/journals

CHEMICAL COMPOSITION, ANTIOXIDANT PROPERTIES, AND MICROBIAL CONTENT OF CHICKPEA (*Cicer arietinum* L.) STEEP LIQUOR

Ahmed M. Saad*, R.A. Elmassry and M.F. Ramadan

Agric. Biochem. Dept., Fac. of Agric., Zagazig Univ., Egypt

ABSTRACT

The aim of this work was to investigate the chemical composition, antioxidant properties and microbial content of chickpea (*Cicer arietinum* L.) steep liquor. 250 g of chickpea seeds were submerged soaked in 500mL boiled sterilized distilled water (1:2 w/v) for 24 h at 37°C, then the filtration was run on Whatman paper No1. As results, 8.2 g of freeze-dried CHSL were obtained. Lysine was the main free amino acid accounted for 77% of total free amino acids followed by serine (6.49%). The results of total amino acids found in CHSL indicated that arginine was the main amino acid accounted for 24% of total amino acids followed by tyrosine (20%). Total carbohydrate in the CHSL was 1.47 % w/w, while the total reducing sugar was 1.25% of total carbohydrates. Levels of nicotinic acid, pyridoxine, thiamin, riboflavin, folic acid, and vitamin B_{12} were 14.3, 3.14, 24.2, 1.11, 0.59, and 24.5 mg/100mg CHSL, respectively. The results clearly indicated that CHSL exhibited antioxidant activity. In general, scavenging activity was increased with increasing the fermentation time. After 24 h fermentation, scavenging activity of CHSL reached 94% while tertiary butyl hydroquinone (TBHQ) exhibited 96%. Also the antioxidant activity, generally, was increased comparing with TBHQ activity, while the highest value noticed after 16 h compared with TBHQ (82 and 82) respectively. Twenty-five Bacilli isolates were separated from freezed-dried CHSL on nutrient broth medium. According to the results it could be concluded that CHSL is might be used as an alternative to yeast extract for syngas fermentation because it is rich in nutrients and lower in cost compared to yeast extract.

Key words: Chickpea, chickpea steep liquor, chemical composition, antioxidant activity and chickpea microflora.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the oldest and most widely consumed legumes in the world; it is a staple food crop in some tropical and subtropical countries. Chickpea seeds are a cheap source of high quality protein in the diet of millions in the developing countries. In addition, they are also a good source of carbohydrates, minerals, and trace elements. Chickpea is considered a healthy vegetarian food and it is one of the most important human nutrition (Duke, 1981). Chickpea cultivars were grouped into two types: desi (Indian origin) and kabuli (Mediterranean and Middle Eastern origin) (Kaur *et al.*, 2005). Substantial differences in these two groups have been observed with regard to their seed coat percentage, crude fiber content, trace element composition, polyphenol content (Chavan *et al.*, 1986; Jambunathan and Singh 1981), parching properties (Kaur *et al.*, 2005), and properties of their flours (Kaur and Singh 2005). Chickpea has a medical benefit (Pandey and Enumeratio 1993 and Warner *et al.*, 1995).

Steep liquor of some plants is important, cheap and environmental -friendly in many products *e.g.* corn steep liquor (CSL). The development of a low cost fermentation medium alternative containing all essential nutrients required for cell growth and product formation would reduce the overall cost of the

* **Corresponding author: Tel.** : +201098143972
E-mail address: ams411987@yahoo.com.

fermentation process. Standard medium for *Clostridium* strain P11 is composed of yeast extract, vitamins, minerals, trace metals and reducing agent (Saxena, 2008 and Saxena and Tanner, 2010). Apart from the reducing agent, yeast extract (YE) is the most expensive component. Some inexpensive nutrients that could replace YE are CSL, hydrolyzed cottonseed flour, hydrolyzed soy flour and ethanol stillage (Witjitra *et al.*, 1996).

Chickpea is also utilized in either whole or paste form as a main or side dish after cooking (Amr and Yaseen, 1994) or as a snack food after roasting (Koksel *et al*, 1998). It is a rich source of lysine and together with carbohydrates and minerals offers nutritional benefits when added to bread formulations (Estevez *et al*, 1987). In some Mediterranean countries, fermented chickpea is being used as a leavening agent to make traditional breads and rusks. By the addition of fermented chickpea in the wheat flour, besides to the enhancement of the nutritional quality, the product's shelf life is also expanded (Tulbek *et al*, 2003).

According to (Hatzikamari *et al.*, 2007) reducing sugars were observed with the progression of chickpea fermentation. Since the reducing sugars support the growth of indigenous flora they were reduced by almost 50% after 18 h of fermentation due to their consumption by the actively increasing population of clostridia. On the other hand a rapid increase in the total free amino acids was observed, the free amino acid content of the FL increased four times during the first 10 h, when bacilli were actively growing. The degradation of chickpea proteins became obvious after 8–10 h of fermentation; presumably, due to the proteolytic activities of *Bacillus spp.* at the end of fermentation the content of amino acids was not increased.

According to Haiwei (2010) the antioxidant activity of soybean Fra-III was clearly related to the amino acid composition, the content of the total hydrophobic amino acids (HAA) were 30.03%. It was reported that an increase in hydrophobicity (Hφ) of peptides would increase their solubility in lipid and therefore enhance their antioxidant activity. Glu., Arg., Phe., Lys., Leu., Ala. and Asp. were the major constituent amino acids of soybean Fra-III. It was commonly believed that His, Met and Cys. are very important to the radical scavenging activity of peptides due to their special structures of characteristics: the imidazole group in His has the proton-donation ability; Met. is prone to oxidation of the Met sulfoxide; Cys. donates the sulfur hydrogen. These amino acids could favor the radical scavenging properties of soybean Fra-III. Therefore, the amino acids composition might play an important role on its activity.

B vitamins are a group of water-soluble vitamins that play an important role in cell metabolism. The B vitamins were once thought to be a single vitamin, referred to as vitamin B. and they are chemically distinct vitamins that often coexist in the same foods. In general, supplements containing all eight are referred to as a vitamin B complex. In general, the contents of thiamin, riboflavin, and niacin were increased during the fermentation according to (Yang and Zhang 2009).

Changes in enzyme activities and in the chemical composition of the fermenting liquid are caused by indigenous *bacilli* and *clostridia* populations (Hatzikamari *et al.*, 2007). Changes in the chemical constituents of the fermenting liquid can be attributed mainly to *bacilli* until 8–10 h of fermentation and then to clostridia until 18 h. Bacilli (*Bacillus subtilis*) are important starter cultures for alkaline fermentations observed in traditional fermented legumes, such as the Indian Kinema (from spontaneously fermented soybeans), the African Soumbala (from locust beans) and others (Sarkar *et al.*, 2002). However, throughout the fermentation of chickpea extracts, a significant decrease of pH was observed (Hatzikamari *et al.*, 2007). Similarly, during Gergoush-making from milk, lentils, and wheat flour, the dominant bacteria are bacilli, clostridia and lactic acid bacteria, and the pH of the fermenting product is reduced (Sherfi and Hamad 2001).

Also fermented chickpea liquid was used instead of baker yeast in preparing some bakeries, such as white breads, white loaf bread, flat tannour bread and lavash (Rhegag) bread which prepared from white flour using fermented chickpeas infusion (ca'ak starter) instead of the classical starter (Baker yeast).

The lavash prepared from classical starter had higher evaluation scores. According to Al - Khafaji *et al.* (1999), types of flat bread had higher scores for most characters studied when fermented chickpeas infusion used as starter. Some characters had some reduction levels of evaluation scores of the last types of bread but this reduction was statically non-significant.

The aim of this work was to investigate the chemical composition, antioxidant properties and microbial content and of fermented chickpea liquor.

MATERIALS AND METHODS

Materials

Plant materials

Chickpea (*Cicer arietinum* L.) were purchased from local market, Zagazig City, Sharkia Governorate, Egypt.

Chemicals

Tryptic Soy Agar was purchased from Biolife, Milano-Italia. DPPH, isopropanol, hydrochloric acid, and sodium hydroxide were obtained from Merk (Germany). All chemicals used in the experiments were of analytical grade.

Methods

Processing treatments

The seeds were hand-sorted to remove wrinkled, moldy seeds and foreign material, then stored in polyethylene bags in the refrigerator (4°C) until needed.

Soaking

Chickpea seeds (250g) were submerged soaked in 500 mL boiled distilled water (1:2, w/v) for 24 h at 37°C according to Badshah *et al.* (1991). After 24 h, foam was formed and the beaker was taken from incubator. In another 500 mL beaker, filtration was run on Whatman paper No.1 and chickpea steep liquor (CHSL) about (300 mL) was obtained.

Hydrolysis

HCl (2.5%) was added to 50 mL CHSL in an oven at 100°C for 12 h then the hydrolysate was filtered using Whatman No.1 paper, to obtain hydrolysate chickpea steep liquor's (HCHSL) with pH 1.3. pH was adjusted using 2.5% NaOH to 4.7. HCHSL was filtered at pH 4.7 and the filtrate pH was adjusted again to pH 7 using 2.5% NaOH. The obtained HCHSL and CHSL were freeze-dried using Lyophilizer (Thermo Fisher scientific further analysis).

Analytical Methods

Chemical Composition of CHSL

Sugars

The CHSL was extracted with 80% ethanol, centrifuged at 2.200-x g and the amount of reducing sugars were determined calorimetrically by the method of Miller (1959) .Three mL of the clear ethanolic extract were mixed with 3 mL of DNSA reagent (3,5-dinitrosalicilic acid 1.0% w/v in 1.0% w/v NaOH, 0.2% w/v phenol and 0.05 w/v Na_2SO_3 added just before the use), and boiled at 95°C for 15 min, then 1 mL of 40% (w/v) potassium-sodium tartrate solution was added. After cooling, the absorbance measured at 575 nm. A standard curve was made with known concentrations of glucose. The amount of reducing sugars finally expressed as mg of glucose/mL of CHSL, while total carbohydrates were determined according to Hedge and Hofreiter (1962).

Vitamins

B vitamins are a group of water-soluble vitamins that play important roles in cell metabolism. Vitamins were determined using HPLC according to the method of Batifoulier *et al.* (2005). The liquid chromatography system consisted of a Alliance 2960 Separation Module (Waters, Saint Quentin en Yvelines, France) with a Multi λ fluorescence detector (Waters 2475 The column (15 cm x 4 mm) and the precolumn (2cm x 4mm) were packed with a RP-amide C16 stationary phase with a particule size of 5 µm (Supelco, USA). The column and the guard column placed in an oven at 30° .C. The mobile phase was potassium phosphate buffer (50mM, pH 6). Methanol (80/20, v/v) delivered at flow rate of 1 ml/min. The injection volume was 20 µl and the duration of the analytical run was 10 min. Fluorescence detection operated at 366 nm excitation and 435 nm emissions.

Amino acids

Free and total amino acids (AA) were determined in CHSL and HCHSL using an INGOS Ltd AAA 400 automatic amino acid analyzer. Acid hydrolysis was carried out according to method of Block *et al* (1958). The freezed-dried sample (100 mg) was hydrolyzed with 6N HCl (10 ml) in a sealed tube at 110°C in an oven for 24 h. The excess of HCl was then freed from 1 ml hydrolyzed under vacuum with occasionally addition of distilled water, then evaporated to dryness. The HCl free residue was dissolved in extract (2ml) of diluting buffer (pH2.2). The buffer was used for dilution of both samples and standards to required concentration.

Antioxidant Activity

DPPH Radical-scavenging activity

10 g of chickpeas were submerged soaked in 20mL boiled distilled water and immediately was filtrated on whatman paper No.1 and chickpea steep liquor (CHSL) about (10 mL) was obtained, the experiment was repeated at intervals of 8, 16, 24 h at room temperature and 37°C.

The electron donation ability of a various fermented stages of CHSL was measured by bleaching of the purple colored solution of DPPH according to the method of Hanato *et al.* (1988). Three hundred µL of each submerged liquor (300 mg extract/1 mL solvent) was added to 3 mL of 0.1 mM DPPH dissolved in methanol. After incubation period of 30, 60 and 120 min at room temperature, the absorbance was determined against a control at 517 nm (Gulcin *et al.*, 2004). Percentage of antioxidant activity of free radical DPPH was calculated as follow:

Antioxidant activity (Inhibition) %

$$= [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of CHSL. TBHQ was used as a positive control. Samples were analyzed in triplicate.

Carotene/linoleic acid bleaching

The ability of CHSL and synthetic antioxidants to prevent the bleaching of β-carotene was assessed as described by Keyvan *et al.* (2007). In brief, 0.2 mg of β-carotene in 1 mL of chloroform, 20 mg of linoleic acid and 200 mg of Tween 20 were placed in a round-bottom flask. After removal of the chloroform, 50 mL of distilled water were added and the resulting mixture was stirred vigorously. Aliquots (3mL) of the emulsion were transferred to tubes containing CHSL or synthetic antioxidant. Immediately after mixing 0.5 mL of extract solution (300 mg extract /1 mL solvent), an aliquot from each tube was transferred to a cuvette and the absorbance at 470 nm was recorded (Abs^0). The remaining samples were placed in a water bath at 50°C for 2 h, then the absorbance at 470 nm was recorded (Abs^{120}). A control without extract was also analyzed. Antioxidant activity was calculated as follows:

Antioxidant activity (%)

$$= [1-(Abs^0 sample - Abs^{120} sample)/(Abs^0 control - Abs^{120} control)] \times 100$$

Where Abs^0_{sample} is the absorbance of sample at 0-time, Abs^{120}_{sample} is the absorbance after 120 min, $Abs^0_{control}$ is the absorbance of control at 0-time, and $Abs^{120}_{control}$ is the absorbance of control after 120 min.

Isolation and Identification of Microorganisms

Microbiological media used (Jacob and Gerstein 1960). A medium used to determine the total bacterial count had the following components (g/1000 mL tap water):

Peptone	5.0
Beef extract	3.0
Agar	20

Moreover, pH was adjusted to 7.0

Isolation of Bacterial Fermentations

One gram of freeze-dried CHSL was suspended in 9 mL sterilized water in conical flask (100 mL), thoroughly shacked for 10 min and serial dilution series up to 10^{-7} were prepared. Plates incubated at 30°C for 2 days, then individual colonies picked up and the purified isolates maintained on nutrient agar at 4°C.

Counting methods used for each microbial group

Isolates were counted according to Dilution-plate count where total count, spore formers, total proteolytics and total saccharolytics were

incubated at 30°C /2-3 days on plate count agar, milk agar and glucose agar supplemented with bromocresol purple Harrigan (1998) and Benson (2001).

Screening of bacterial isolates according to fermentation

Acid gas production test was performed according to the method described by Seeley and Vandemark (1970). Bacterial isolates were tested for acid and gas production by inoculating 5 ml of the sterile glucose broth with bromocresol purple (15 ml /l of 0.04% solution as pH indicator) in test tubes containing Durham's tube. The tubes were incubated for seven days at 30°C. Accumulation of gas in Durham's tube was taken as positive for gas production. The change in color of the medium to yellow was taken as positive for acid production.

Casein hydrolysis was performed according to the method described by Seeley and Vandemark (1970). Petri plates of skim milk agar were streaked with test cultures and incubated at 30°C for observing clear zone against black background it was taken as positive for acid production.

Identification of best isolates

Bergey manual and Biolog system number 21124 Cabot Blvd. Hyward CA 94545 (2004) in Cairo mercin was used for identification the most active isolates according to Klingler *et al.* (1992).

RESULTS AND DISCUSSION

Chickpea, one of the oldest and most widely known legumes in the Middle and Far East, stands as a promising raw material for the preparation of fermented food products. Fermentation brings about desirable changes in legume seeds, such as elimination of off flavors, improvement in digestibility, and enhancement in keeping quality and safety, and better nutritional value.

Results indicated that 8.2g of freeze-dried CHSL were obtained from 250 g of chickpea (3.28 % w/w) after 24 h of fermentation at 37°C.

Amino Acid Composition

The free amino acid content of the CHSL was recorded using amino acid analyzer. Table 1 represents the results of free amino acids found in CHSL after 24 h. Lysine was the main amino acid accounted for 77.13% of total free amino acids followed by serine (6.49%). Other amino acids were found in lower amounts.

Data in Table 2 refer to the results of total amino acids found in CHSL after 24 h fermentation . Argenine was the main amino acid accounted for 24.31% of total amino acids followed by tyrosine (20.12%). Other amino acids were found in descending amounts or as traces.

Sugar Composition

Data in Table 3 showed that the results of total carbohydrate in the CHSL after 24 h fermentation was 1.47 % w/w, while the total reducing sugar was 1.25%.

Vitamins Composition

Individual B vitamin supplements are referred to the specific name of each vitamin (e.g., B_1, B_2, B_3 etc.). The obtained results in table 4 indicated that the levels of nicotinic acid, pyridoxine, thiamin, riboflavin, folic acid, and vitamin B_{12} were 14.3, 3.14, 24.2, 1.11, 0.59, and 24.5 mg/100mg CHSL, respectively.

Antioxidant Properties of CHSL

DPPH radical-scavenging activity

Antioxidants react with DPPH$^{\cdot}$, reducing the number of DPPH$^{\cdot}$ free radicals to the number of their available hydroxyl groups. Therefore, the absorption at 517 nm is proportional to the amount of residual DPPH$^{\cdot}$ (Juan *et al.*, 2005). It is visually noticeable as a discolouration from purple to yellow. The scavenging activity of the tested extracts against DPPH$^{\cdot}$ was concentration-dependent. The results of DPPH radical-scavenging activities of various fermented stages of CHSL are represented in Fig 1. The results clearly indicated that CHSL exhibited antioxidant activity. In general, scavenging activity of CHSL increased with increasing the fermentation time. After 24 h fermentation, AA of CHSL reached 94% while tertiary butyl hydroquinone (TBHQ) exhibited 96%.

It has been proven that the antioxidant activity of various fermentated stages of CHSL is mainly ascribable to the concentration of phenolic compounds in the plant (Heim *et al.*, 2002). The results of the DPPH$^{\cdot}$ free radical

scavenging assay suggest that components involving CHSL are capable of scavenging free radicals *via* electron- or hydrogen-donating mechanisms and thus might be able to prevent the initiation of deleterious free radical mediated chain reactions in susceptible matrices.

Carotene /linoleic acid bleaching assay

Oxidation of linoleic acid produces hydroperoxide-derived free radicals that attack the chromophore of β-carotene, resulting in bleaching of the reaction emulsion. An extract capable of retarding/inhibiting the oxidation of β-carotene may be described as a free radical scavenger and primary antioxidant (Liyana-Pathirana and Shahidi 2006). As can be seen in Fig 2, CHSL at different fermentation periods were capable of inhibiting the bleaching of β-carotene as antioxidant, where antioxidant activity of CHSL was highest after 16h (82) comparing with TBHQ activity (82). The results revealed that, overall; CHSL had comparable scavenging ability to the synthetic antioxidants TBHQ.

Isolation and Identification of Microorganism in CHSL

Isolation

Twenty-five isolates were separated from freeze-dried CHSL on nutrient broth medium (Jacob & Gerstein, 1960). Fifteen isolates were produced gas in test tubes containing Durham's tube, after incubation for 7 days at 30°C. Ten isolates were changed the color of the medium to yellow and caused casein hydrolysis.

Counting

Data in Table 5 showed that the total count of microorganisms found in CHSL after 24 h fermentation.

Identification

All the check isolates (identified by Bergeys manual and Biolog technique) formed completely white, round, smooth and shiny colonies. During microscopic examination all, the isolates were found to be gram positive long rods. Presence of endospores was confirmed by endospore staining. The ability of sugars and proteins analysis represented in Table 6 according to Bergey (1994).

The results were confirmed by identification using Biolog system Klingler *et al.* (1992) (Cairo MIRCEN, Faculty of agriculture, Ain Shams University).

According to the results it could be concluded that CHSL is might be used as an alternative to yeast extract for syngas fermentation because it is rich in nutrients and lower in cost compared to yeast extract.

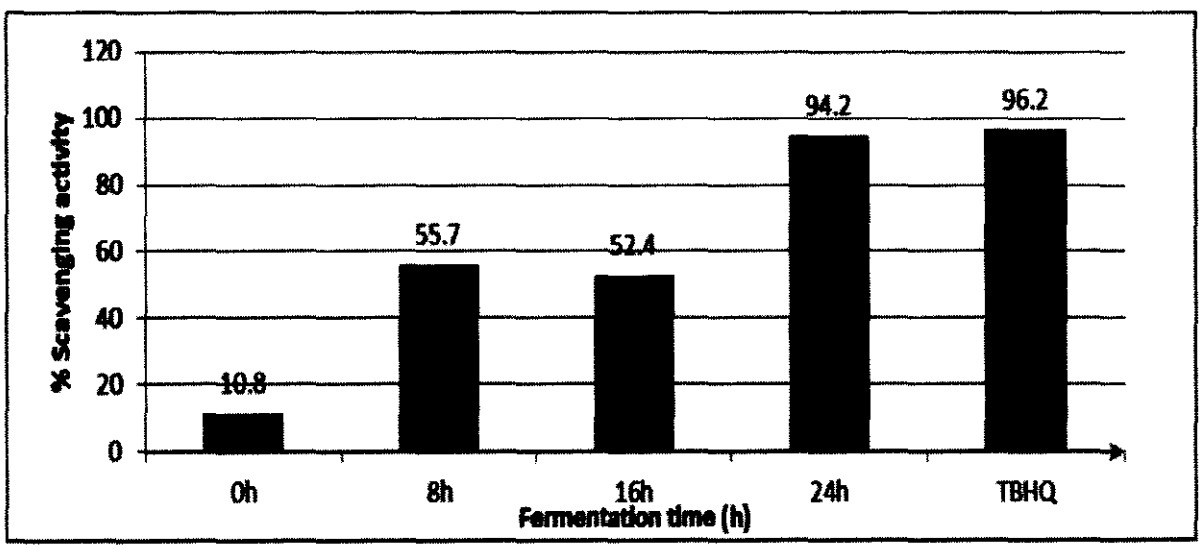

Fig. 1. Scavenging activity of CHSL against DPPH˙ free radical compared with TBHQ

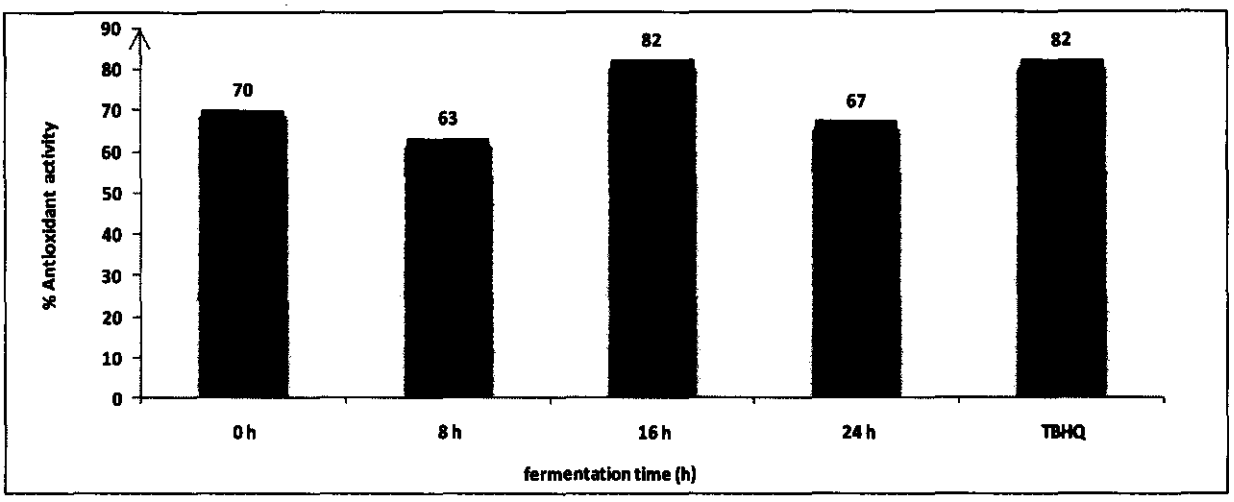

Fig. 2. Antioxidant activity of CHSL in β-carotene/linoleic acid system compared with TBHQ

Table 1. Free amino acids composition of CHSL after 24 h fermentation

Amino Acid	%	g/100ml
Ser	6.49	0.1298
Pro	1.16	0.0232
Ala	1.09	0.0218
Cys	1.62	0.0324
Met	1.65	0.0330
Leu	2.99	0.0598
Tyr	3.77	0.0754
His	4.09	0.0818
Lys	77.13	1.5426

Table 2. Total amino acids composition of CHSL after 24 h fermentation

Amino Acid	%	g/100g
Thr	5.437	0.9321
Ser	2.87	0.4879
Pro	2.81	0.4777
Gly	4.198	0.7123
Ala	12.13	2.0604
Val	7.635	1.9271
Leu	3.409	0.5780
Tyr	20.12	3.4238
Phe	2.10	0.3570
His	9.084	1.5436
Lys	5.896	1.0013
Arg	24.31	4.1378

Table 3. Sugars percentage from total carbohydrates of CHSL after 24 h fermentation

Sugars	%
Reducing sugars	**1.25%**
Total sugars	**1.47%***

* From total carbohydrates

Table 4. B vitamins composition of CHSL after 24 h fermentation

Vitamin	mg/100mg
Nicotinic acid	14.3
Pyridoxine	3.14
Thiamin	24.2
Riboflavin	1.11
folic acid	0.59
vitamin B_{12}	24.5

Table 5. The total count of microorganisms found in CHSL after 24 h fermentation

Test	Result
Total plate count(cfu/g)	$1.5x10^3$
Total spore formers (cfu/g)	60
Total proteolytics (cfu/g)	$7.0x10^2$
Total saccharolytics (cfu/g)	$1.2x10^3$

Table 6. Identification of the best isolates found in CHSL after 24 h fermentation

Characterization	*Bacillus megitherum*	*Bacillus subtillus*
Cultural characterization		
Color	White	White
Size	Large	medium
Morpholigcal characterization		
Shape	Long rods cause central cell swelling.	Long rods do not cause swelling.
Mortalty	Mobile.	Mobile.
Endosporme		
Shape	Circle , large	Circle
Location	Center	center
Stanning		
Gram	G+	G+
Acid fast	Not resistant.	.Not resistant
Endospore	Center, circle, large.	Center, circle.
Utilitation of diffirent carbon source		
No carbon	-	-
Glucose	+	+
Lactose	+	+
Sucrose	+	+
Fructose	+	+
Galactose	+	+
Utilitation of diffirent nitrogen source		
Pepton	+	+
Potassium nitrate	+	+
Beef extract	+	+
Yeast extract	+	+
Glatien	+	+
Casin	±	+
Optimal temperature for growth		
20°C	-	-
25°C	-	-
30°C	+	+
37°C	-	-
50°C	-	-
Oxygen recoirments		
Aerobic	+	+
Anaerobic	-	-
Facultative	-	-
Microaerophilic	-	-

REFERENCES

Al-Khafaji, Z.M., S.S. Abdul-Hussien and R.A. Awadala (1999). Using of fermented chickpea infusion in some bakeries.Iraqi J. of Agric. Sci., 30:523-530.

Amr, A.S. and E.I. Yaseen (1994). Thermal processing requirements of canned chickpea dip. Inter. J. of Food Sci. and Tech., 29: 441-448.

Badshah, A., Zeb, Anurang and A. Sattar (1991). Effect of soaking, germination and autoclaving on selected nutrients of rapeseed. Pakistan J of Sci. and Industrial Res., 34, 446–448.

Batifoulier, F., M.A. Verny, C. Besson, C. Demigne and C. Remesy (2005). Determination of thiamine and its phosphate esters in rat tissues analyzed as thiochromes on a RP-amide C16 column. J. Chromatogr. B 816 (2005) 67–72.

Benson, H.J. (2001). Microbiological applications laboratory manual laboratory manual for general microbiology. Eighth Edition. The McGraw-Hill Companies, New York.

Bergey (1994). Manual of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore ISBN 0-683-00603-7.

Block, R.J., E.L. Durrum and G. Zweig (1958). Annual of paper chromatography and paper electrophoresis 2nd, ed.

Chavan, J.K., S.S. Kadam and D.K. Salunkhe (1986). Biochemistry and technology of chickpea (*Cicer arietinum* L.) seeds. CRC Critical Reviews in Food Science and Nutrition, 25: 107–132.

Duke, J.A. (1981). Handbook of legumes of world economic importance. New York, USA: Plenum Press, 52–57.

Estevez, A.M. F. Figuerola, M. Vasquez, E. Castillo and E. Yanez (1987). Arch Latinoam Nutr 37:515–5244 .Tulbek MC, Hall C, Schwarz JG (2003) Abstract in IFT Annual Meeting, Chicago, 13–16.

Glucin, I., O. I. Kufrevioglu, M. Oktay, and M. E. Buyukokuroglu, (2004). Antioxidant, antimicrobialm antiulcer and analgesic acticites of nettle (*Urtica dioica* L.). J. Ethnopharmacology. 90:205-215.

Haiwei, R. (2010). Antioxidant and free radical-scavenging activities of black soybean peptides (BSP). Int J Agric & Biol Eng. 3, (2): 64-69.

Hanato, T., H. Kagawa, T. Yasuhara and T. Okuda (1988). Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem. Pharm. Bull., 36: 2090–2097.

Harrigan, W.F. (1998). Laboratory methods in food microbiology. Academic Press, San diego.

Hatzikamari, M., D.A. Kyriakidis, N. Tzanetakis, C.G. Biliaderis and E. Litopoulou-Tzanetaki (2007). Biochemical changes during a submerged chickpea fermentation used as a leavening agent for bread production. European Food Research and Technology. 224: 715–723.

Hedge, J.E. and B.T. Hofreiter (1962). In: Carbohydrate Chemistry, 17 (*Eds.* Whistler R.L. and Be Miller, J.N.), Academic Press, New York.

Heim, K.E., A.R. Taigliaferro and D.J. Bobilya (2002). Flavonoid antioxidants: chemistry, metabolism and structure–activity relationships. J. Nutr. Biochem.13: 572–584.

Jacob, M.B. and M.J. Gerstein (1960). Hand book of microbiology. D. Van Nostrand Co. Inc. Princeton. New Jersey, (USA).

Jambunathan, R. and U. Singh (1981). Studies on desi and kabuli chickpea (*Cicer arietinum* L.) cultivars 3. Mineral and trace elements composition. J. of Agric. and Food Chem., 29, 1091–1093.

Juan, X., C. Shubing and H. Qiuhui (2005). Antioxidant activity of brown pigment and extracts from black sesame seed (*Sesamum indicum* L.). Food Chem., 91: 79–83.

Kaur, M. and N. Singh (2005). Studies on functional, thermal and pasting properties of flours from different Chickpea (*Cicer arietinum* L.) cultivars. Food Chemistry, 91(3): 403–411.

Kaur, M., N. Singh and N.S. Sodhi (2005). Physicochemical, cooking textural and roasting characteristics of Chickpea (*Cicer arietinum* L.)Cultivars. Journal of Food Engineering, 69: 511–517.

Keyvan, D.H.J., D. Damien, L. Into and H. Raimo (2007). Chemical composition and antioxidative activity of Moldavian balm (*Dracocephalum moldavica* L.) extracts. LWT – Food Sci. Technol., 40: 1655–1663.

Klingler, J.M., R.P. Stowe, D.C. Obenhuber, T.O. Groves, S.K. Mishra, D.L. Pierson (1992) Evaluation of the Biolog automated microbial identification system. Appl Environ Microbiol. 58(6): 2089–2092.

Koksel, H., D. Sivri, M.G. Scanlon and W. Bushuk (1998). Comparison of physical properties of raw and roasted chickpeas (leblebi). Food Research International. 31: 659-665.

Liyana-Pathirana, C.M. and F. Shahidi (2006). Antioxidant properties of commercial soft and hard winter wheats (*Triticum aestivum* L.) and their milling fractions. J. Sci. Food Agric., 86: 477–485.

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31: 426–428.

Pandey, G. and G. Enumeratio (1993). Gyanendra PlantaMedica Ausadhiya Padapavali. Indian medical science series Delhi, India: Spring, vol. 15 p. 116.

Sarkar, P.K., B. Hasenack and M.J.R. Nout (2002). Diversity and functionality of Bacillus and related genera isolated from spontaneously fermented soybeans (Indian Kinema) and locust bean (African Soubala). Inter. J. Food Micro., 77: 175–186.

Saxena, J. (2008). Development of an Optimized and cost-effective medium for ethanol production by clostridium strain P11. PhD, University of Oklahoma‹ Norman, OK, 110.

Saxena, J. and R.S. Tanner (2010). Effect of trace metals on ethanol production from synthesis gas by the ethanologenic acetogen, Clostridium ragsdalei. Journal of Industrial Microbiology and Biotechnology. Doi: 10.1007/s10295-010-0794-6.

Seeley, H.W. and P.S. Vandemark (1970). Microbes in action; A laboratory manual of microbiology. D.B. Tarapogevale Sons and Company Limited. Bombay.pp:86-95.

Sherfi, S.A., and S.H. Hamad (2001). Microbiological and biochemical studies on Gergoush fermentation. Inter. J. Food Micro. 76: 247–252.

Tulbek M.C., C. Hall and J.G. Schwarz (2003). Abstract in IFT Annual Meeting, Chicago, 13–16.

Warner P.K.W., V.P.K. Nambiar and C. Remankutty (1995). Indian medicinal plants. Chennai, India: Orient Longman; 773–774.

Witjitra, K., M.M. Shah and M. Cheryan (1996). Effect of nutrient sources on growth and acetate production by *Clostridium thermoaceticum.* Enzyme and Microbial Technology 19 (5): 322–327.

Yang, H. and L. Zhang (2009). Changes in some components of soymilk during fermentation with the basidiomycete *Ganoderma lucidum.* Food Chem., 112:1–5.

التركيب الكيميائي والخصائص المضادة للأكسدة والمحتوى الميكروبي لماء نقع الحمص

احمـــد محمـــد ســعد – رجب عبـد الفتــاح المصري- محمد فوزي رمضان حساتين

قسم الكيمياء الحيوية الزراعية – كلية الزراعة – جامعة الزقازيق

الهدف من هذا البحث دراسة التركيب الكيميائى والخواص المضادة للاكسدة والمحتوى الميكروبى لماء نقع الحمص. طبقا للنتائج المتحصل عليها فان ٨,٢ جم نتجت عن تجفيد ماء نقع ٢٥٠ جم حمص على درجة ٣٧°م لمدة ٢٤ ساعة (٣,٢٨% وزنية / وزنية). وقد مثل الليسين ٧٧% من الاحماض الامينية الحرة لذلك فهو الحمض الامينى الرئيسى يليه السيرين بنسبة ٦,٤٩%. بينما الارجنين هو الحمض الاساسى في الاحماض الامينية الكلية في ماء نقع الحمص بعد ٢٤ ساعة تحضين بنسبة ٢٤% يليه التيروزين بنسبة ٢٠% من الاحماض الامينية الكلية. وكانت نسبة السكريات الكلية بعد ٢٤ ساعة تحضين في ماء نقع الحمص ١,٤٧% بينما نسبة السكريات المختزلة الكلية ١,٢٥% من الكربوهيدرات الكلية. وكانت كميات الفيتامينات الذائبة في الماء (حمض النيكوتينك، البيريدوكسين، الثيامين، الريبوفلافين، حمض الفوليك وفيتامين ب ١٢) بالمجم/١٠٠مجم في ماء نقع الحمص هي: ١٤,٣، ٣,١٤، ٢٤,٢، ١,١١، ٠,٥٩ و٢٤,٥ على الترتيب. كما أوضحت النتائج ان ماء النقع له خواص مضادة للأكسدة حيث وجدت علاقة طردية بين هذه الخواص وفترة التحضين. حيث وصلت نسبة التخلص من الشقوق الحرة لمنقوع الحمص بعد ٢٤ ساعة تحضين ٩٤% مقارنة بمضادات الاكسدة الصناعية (TBHQ) حيث كانت ٩٦% كما وصلت نسبة التضاد للاكسدة بعد ١٦ ساعة تحضين ٨٢% مقارنة بمضادات الاكسدة الصناعية (TBHQ) حيث كانت ٨٢%. تم عزل ٢٥ سلالة من الميكروبات العصوية (Bacilli) على بيئة المرق المغذى وتم تعريف أكفأ سلالتين من حيث انتاج الحموضة والغاز. لذلك طبقا للنتائج يمكن الاستنتاج انه يمكن استبدال الخميرة بماء نقع الحمص حيث انه ذو قيمة غذائية عالية وتكاليف اعداده بسيطة لا تقارن بمستخلص الخميرة.

المحكمون:

١- أ.د. نجـــاح الشـــحـــات علـــى أستاذ الكيمياء الحيوية – كلية الزراعة – جامعة عين شمس.

٢- أ.د. على محمد على إبراهيم الجابى أستاذ الكيمياء الحيوية – كلية الزراعة – جامعة الزقازيق.