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EFFECT OF *Jerusalem artichoke* AND *Tigernut* AS SUGAR SOURCES ON PATHOGENIC and PROBIOTIC BACTERIA GROWTH IN SOME FUNCTIONAL FOOD (BIO - YOGHURT AND WHEAT DOUGH)

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ABSTRACT

In recent time, there has been an increase interest to improve prebiotic material and probiotic bacteria mainly in food. This study aims to evaluate the effect of *Jerusalem artichoke (JR)* and *Cyprus esculents* (Tigernut or Chufa) (TN) as prebiotics source, to improve growth and survival of probiotic bacteria in yoghurt and as a preserved agent against the food borne bacteria *in vitro*. In addition, emphasis on improve nutritional value of dough using a new source of inulin by supplemented wheat flour with *JR* and *TN*. In *vitro* JR and TN were used as prebiotic for evaluation their effect as growth promoters for prebiotic bacteria (*Lactobacillus plantarum*, *Lactobacillus curvatus* and *Bacillus subtilis*) and evaluated antimicrobial activity against *Staphylococcus aureus* at different concentrations (5.0 and 10.0%). The results revealed that JR and TN contained of a sufficient amount of macronutrients carbohydrate, fiber and valuable amino acids. Sugar profile of these plants showed that, inulin was the most abundant polysaccharides in JR while TN was abundant in fructose. These polysaccharides (inulin and fructose) as considered as bioactive ingredients and prebiotic compounds. In addition, 10 % supplementation level of JR or TN resulted in greater growth rates of prebiotic bacteria, as well as showed stronger antimicrobial activity. Therefore, these results could suggest a preferential utilization of *JR* and *TN* in the people diet for improving the nutritional value and provide health benefits as functional foods, as well as considered economic and natural antimicrobial agent in food preservation.

Keywords:*Jerusalem artichoke*, Tigernut, Inulin, Yoghourt, Wheat dough, Prebiotic, Antimicrobial,

INTRODUCTION

Probiotic foods including dairy products defined as food containing live microorganisms, which believed to enhance health by improving the balance of microflora in the jut (Tamime *et al.* 2005). The microorganisms that most commonly used as probiotics belong to the heterogeneous group of lactic acid bacteria and the genius Bifidobacterium. Prebiotics are non-digestable components of functional food that stimulate the proliferation and activating of bacterial population desirable in the colon and inhibit pathogen multiplication, hence beneficially acting on the host (Mattila *et al.* 2002). The most important prebiotics are fructans (inulin and oligofructoses), glucans and mannans, which are soluble and fermentable fibers (Gibson *et al*. 2004). Supplementation of skim milk with inulin, even at a low concentration significantly improves the growth and viability of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* and *Bifidobacterium lactis* **(**Cruz *et al*. 2010 and Oliveria 2011). Inulin is among the most famous prebiotic compounds. Also

when inulin added to the food in low concentrations the rheological properties and sensory quality of the product improve (Aguilar *et al*. 2015). *Cyperus esulentus*, root stock snack or earth almond is among the popular, cheap and sweet convenience foods in Africa. It has been cultivated since the fourth millennium BC in Egypt, and for several centuries in Southern Europe. It is a perennial tuber commonly found in Egypt. The major components of this tuber are complex carbohydrates. It is comprise fructosyl-fructose linked compounds such as inulin. In human nutrition intervention trials, inulin appeared to be more effective than oligofructose in reducing triglyceridemia, whereas in animals (especially in rats), both products were equally active. In the high fat diet HF and the diets, containing inulin delayed the lowest plasma triglyceride and total cholesterol levels (Reimer and Russell 2008). In young adolescents, daily consumption of a combination of prebiotic short- and long-chain inulin-type fructans significantly increases calcium absorption and enhances bone mineralization during pubertal growth. Effects of dietary factors on calcium absorption may be modulated by genetic factors, including specific vitamin D receptor gene polymorphisms (Abrams *et al.* 2005).

Among other plants rich inulins is *Jerusalem artichoke* (*Helianthus tuberosus L*.) (JR), its tuber accumulates similar levels of inulin (10-20 %) of fresh tuber). Therefore, this study aims to evaluate effect of JR and TN as prebiotics source in fermented dairy products and assess their antimicrobial effect. In addition, improve nutritional quality of bake product by new source of fructosan.

MATERIALS AND METHODS

Preparation of samples:

Jerusalem artichoke (JR) was kindly obtained from farm from Sharkia Egypt. Brown *Cyperus esculents* (tigernut tubers or Chufa) (TN) was bought from local market (Sharkia Egypt). TN they screened on a metallic sieve in order to calibrate them according to their average diameter (two sieve sizes were used 1 and 0.5 cm), then washed with tap water to remove sands and other undesirable materials. Also *Jerusalem artichoke* was sliced cutting (think 0.5 cm) and dried on oven drying at 50 °C until dry, then ground into flour using attrition mill (Globe , China) and passed through a 0.45 mm mesh size sieve, packaged in an airtight polyethylene bag and stored in a plastic container with lid and then stored in a freezer at -18 °C until analysis.

Soaking experiments:

Soaking experiments on TN were conducting according to Turhan *et al*. (2002). Beakers (500 ml) containing 400 ml distilled water were placed in a constant temperature water bath at 25 °C. During soaking, tubers periodically removed, superficially dried with a tissue paper. The experiment was terminated when tuber moisture content attained an equilibrium value, i.e., when the increment change in sample weight was less than 0.01 g. At least three experiments conducted for every tiger nut diameter at soaking temperature. After soaking TN was drained, rinsed, and ground into flour using attrition mill (Globe, China). The flour samples were passed through a 0.45 mm mesh size sieve. It was then packaged in an airtight polyethylene

bag and stored in a plastic container with lid and then stored in a freezer at) -18 °C from where samples were taken for analysis.

Chemical composition of JR and TN:

Fat, protein, ash, moisture and fiber contents were determined by AOAC (2012), while carbohydrates were obtained by difference.

Determination of amino acids (AA):

It performed according to method described in AOAC (2012). Samples were analyzed for AA using Amino Acid Analyzer (BIOCHROM 30, serial 103274, with EZ chrom manual 2004), software used for data collection and processing. The results performed by percentage of total crude protein. Determination of tryptophan carried out using method described by Miller (1967) after hydrolysis of samples with barium hydroxide.

The predicted protein efficiency ratio (P-PER) was calculated using the equation according to Alsmeyer *et al*. (1974): P-PER = -0.468 + 0.454(Leu) – 0.105(Tyr)

Determination of fatty acids of plants and bio-yoghourts:

Samples of each were dried at 60 °C and ground in a coffee grinder to a particle size of approximately 1 mm^3. Fats were extracted from the samples with petroleum ether (60-80 °C boiling fraction) in a Soxtec apparatus (FOSS Tecator, Auckland, NZ). Fatty acids in the extracted oils were esterified by BF_3 and methanol into fatty acid methyl esters (FAMES) according to described method of AOAC (2012). The fatty acid methyl esters analyzed by gas liquid chromatography (Shimidazu GC 2010) using DB-wax column. The carrier gas was Helium with a flame ionization detector, fatty acids identified according to standard FAME.

Determination of polysaccharides:

Determination of polysaccharides in JR was done using the method of (Cabezas *et al.* 2002), 4 grams of sample were dissolved in HPLC grade water sonicated for one hour, centrifuged for 10 min at 4000 Xg, the supernatant were quantitive transferred to measuring flask 100 ml and completed to the mark HPLC grade water sonicated again for 15 min, then poured through 0.20 µm membrane filter. While in TN, using described method by Mano *et al*. (2009) and Helle *et al*. (2010), was used 1 g of sample were dissolved in HPLC grade water sonicated for one hour, centrifuged for 10 min at 4000 Xg the supernatant were quantitate transferred to measuring flask 100 ml and completed to the mark HPLC grade water sonicated again for 15 min. Poured through 0.20 µm membrane filter completed to volume with acetonitrile. Chromatographic analysis of the sugars in JA and TN were determined by HPLC, fitted with RI detector, column typr Rezex 300× 7.8 mm, at 80°C, flow rate 0.6 ml /min with water 100% as mobile phase according to Javier *et al*. (2011).

Determination of vitamins:

Vitamin B1 (thiamine), B2 (riboflavin) were measured as described by Bognar (1992), Beckman HPLC , injector and data handling item Perkin-Elmer flourencece detector LC240 and C18 column 25cm × 4.6 mm were used. Vitamin E (α–tocopherol) was measure by using HPLC according to AOAC (2012), determination was done by using isopropanol n-heptane,

Spectrophotometer detection at 285 nm. Vitamin C was assessed according to the method of Campos *et al.* (2009), briefly homogenized samples (about 1 g) were added to 15 ml of extraction solution (3% metaphosphoric acid, 8% acetic acid, 0.3 N sulfuric acid and 1 m M EDTA). After filtrate under vacuum, it diluted in ultrapure water and adjusts the volume to 25 ml, then centrifuged at 1789 g for 15 min. The supernatant was stored in a refrigerator at 5 °C and analysis by using HPLC.

Determination of minerals:

Mineral contents in JR and TN identified as described by AOAC (2012). The minerals were determined by digesting 0.5 g sample in concentrated HNO3 at a temperature of 85 °C and then in HClO4 at temperature of 180 °C until 1-2 ml of digested samples left. The digested samples then filtered and volume made up to 25 mL. These samples run through an Atomic Absorption Spectrophotometer (Varian, AA240, and Victoria, Australia) using air acetylene flame to determine the minerals content.

Extraction and identification of compounds by GC–MS in methanol extract:

The GC–MS instrument used to separate and detect methanol extracting JA and TN according to the method described by Boskou (2005) and AOAC (2012). One gram of flour was extracted three times with methanol l2 ml. The extracted combined and methanol evaporated under reduced pressure. The residue was dissolved in acetonitrile (2ml) and washed two times with hexane (3ml). Acetonitrile evaporated under vacuum and the residue dissolved in methanol (1ml). Injections of 10µl from this dissolve-extracted lipid in methanol were performed using a GC/MS (Agilent Technologies 6890N computerized system coupled to an MSD, Agilent 5973B mass spectrometer).

Preparation of bio-yoghurt:

The bio-yogurt samples were made from cow milk and prepared as described by Shori and Baba (2011). Samples divided into five groups (100 ml milk /each), JR and TN flours added at 10%. Each of five groups mixtures placed in a glass jars and heated at 85°C for 30 min, then allowed to cool (40 – 42 °C) subsequently inoculated with *Lactobacillus plantarum* and *Lactobacillus curvatus* bacterial cultures at 40°C and fermented until pH 5.7. After incubation, yoghurts were stored in 4°C and examined in order to check the growth of lactic acid bacteria.

Texture profile analysis (TPA):

It was determined by a universal testing machine (Cometech, Btype, Taiwan) provided with software. An Aluminum 25 mm diameter cylindrical probe was used in a TPA double compression test to penetrate to 50% depth, at 1 mm/s speed test. Hardness (N), gumminess, chewiness, adhesiveness, cohesiveness and springiness calculated from the TPA graphic. Firmness (N); maximum force required to compress the sample (was determined as the maximum penetration and expressed in yoghurt), Cohesiveness; extent to which sample could be deformed prior to rupture, Springiness; ability of sample to recover to its original shape after the deforming force was removed, Gumminess; force to disintegrate a yoghurt sample for swallowing (hardness × Cohesiveness) and Chewiness; work to masticate the sample for

swallowing (springiness × gumminess) were determined as described by Bourne (2002).

Organoleptic properties:

Organoleptic properties of bio-yogurt was running after 1 day of refrigerated storage. Ten untrained panels' participants selected randomly for sensory evaluation. Each panel tested two types of bio-yogurt one fortified yogurt with biofidobacterium and JR and second fortified yogurt with biofedobacterium and TN. The evaluation was scored on 1–10 point hedonic scale (1-2 = extremely poor, 3- 4 = poor, 5-6 = fair, 7- 8 = good, 9-10 = excellent) according to taste sour, bitter, sweet, aroma and overall acceptability.

Rheological properties of dough by Alveograph:

White wheat flour used as control (72% extraction ratio), JR and TN /wheat flour blends were prepared at 5%, 10% and 15% of white wheat flour substitutions and wheat. Rheological properties are maintained by Alveograph to determine the quality of wheat flour blends with JR and TN according to AACC (2000) method No (AACC 54-30A). Each Alveograph result was analyzed for the following parameters: Tenacity (P) mm H_2O : the maximum over pressure needed to blow the dough bubble, expresses dough resistance; Extensibility (L) mm : the length of the curve, expresses dough extensibility, Configuration rate (P/L) % : the configuration ratio of the Alveograph curve, Index of swelling (G): index of swelling, Baking strength or (deformation energy (W)10E-4 J: baking strength (surface area of the curve), Elasticity index (Ie) %: elasticity index.

Microbiological evaluation:

Bacterial strains:

Each *Lactobacillus plantarum* code no . (ATCC14917) and *Lactobacillus curvatus* code no. (1136T) strains were obtained from Microbiological Resources Center (Cairo MIRCEN), faculty of Agriculture, Ain Shams University. *Staphylococcus aureus* code no (A.F 15) and *Bacillus subtilis* strains were kindly provided by Dr. Ahmed F. Abdel Salam , Regional Center for Food and Feed, Agriculture Research center, Giza, Egypt).

Isolates maintaining:

Each *L. plantarum* and *L. curvatus* isolates were maintained through monthly transfer on MRS agar, while *Staph aureus* and *Bacillus subtilis* (*B. subtilis*) isolates were maintained through monthly transfer on nutrient agar. All strains were stored at 4 °C.

Standard inoculants:

Standard inoculants were prepared by inoculation of conical flasks (100 ml in volume containing 50 ml of MRS broth pH 5.7) for 24 at 30 °C with loop of *L. plantarum* or *L. curvatus* isolates and another flask containing 50 ml of nutrient broth (pH 6.8) for 24 hr at 30 °C with a loop of *Bacillus subtilis,* beside flask containing 50 ml of buffered peptone water (pH 7.2) for 24 hr at 37°C with loop of *Staph aureus.* Achieve viable cells count were determined by serial dilution and subsequent enumeration on MRS agar for *L. plantarum* and *L. curvatus*, nutrient agar for *Bacillus subtilis* and Vojel Johnson agar for *Staph aureus.* Plant substances of JR and TN were prepared at different

concentrations (5.0 and 10.0 %) and tested as growth promoter for *L. plantarum* and *L. curvatus* activity against *Staph aureus.*

Effect of JR and TN on probiotic bacteria strains in *vitro*:

Erlenmeyer flasks (250 ml) contained 50 ml of MRS broth were inoculated with 1 ml of *L. plantarum* or *L. curvatus* inoculum containing about 10^{13} cfu/ml, then different concentrations (5.0 and 10.0 %) of JR or TN were added to the flask separately, which incubated at 30 °C for 24 hr on rotary shaker (100 rpm). Moreover, flasks contained 50 ml of nutrient broth (pH 6.8) were inoculated with 1 ml of *Bacillus subtilis* inoculum containing about 10^{11} cfu/ml and flasks contained 50 ml of brain heart infusion broth (pH 7.4) were inoculated with 1 ml of *Staph aureus* inoculum containing about 10^{13}cfu/ml then added different concentrations (5.0 and 10.0 %) of JR or TN were added to the flask separately, which incubated at 30 °C for 24 hr on rotary shaker (100 rpm). The control inoculated without any treatment for each bacterial strain at the same experimental condition.

Antimicrobial effect of JA or TN:

Yoghurt samples five treatments as follow control, second and third containing JR, fourth and fifth containing TN at 5.0 and 10.0 % of, respectively. All samples inoculated with *Staph aureus* (about 10^{13}cfu/ml) and inoculated at 37 °C for 3hr, then put at 4°C, the mean cfu/ml for *Staph aureus* was determined according to (Berrang *et al*. 2001).

RESULTS AND DISCUSSION

Chemical composition of JR and TN:

The nutrients and proximate analysis of JR and TN are presented in (Table 1). Results revealed that, JR and TN contained carbohydrate 78.00 and 41.22 % , crude fat 0.38 and 35.43% , ash 5.22 and 4.25 %, fiber 1.33 and 5.64 % and moisture 5.36 and 3.78 %, respectively. Codina-Torrella *et al*. (2014) reported that, the tigernut contain from 25.35 to 28.19% fat and 3.28 to 7.32% protein. The predominant constituent in JR carbohydrates was being 78.00 % and follows by protein being 9.8%. However, the carbohydrate and follows by crude fat, 41.22 and 35.43, respectively, were the most predominate nutrients constituent in TN .

Table 1. Chemical comparison of *Jerusalem artichoke* (JR) and *Tigernut* (TN) /100 g dry base

Nutrients	RDV*	JR		TN	
		Contents**	% of RDV	Contents **	% of RDV
Protein (g)	50	9.8±0.36	19.6	9.7±0.16	19.40
Fat (g)	65	0.38±0.22	0.58	35.43±0.35	54.51
Ash (g)	-	5.22±0.18	-	4.25±0.44	-
Fiber (g)	25	1.33±0.67	5.32	5.64±0.15	22.56
Moisture (g)	-	5.36±0.12	-	3.78±0.65	-
Carbohydrate (g)	300	78.00±0.31	26.00	41.22±0.35	13.74

*** RDV : Relative daily value; from the Food and Nutrition Board (2002) ;**
**** Values represent the mean ±SD of triplicate measurements**

Amino acids content in JR and TN:

Amino acids (AA) content in JR and TN are presented in Table (2). Results revealed that, the protein content of JR and TN containing total essential AA 19.89 and 14.33 g/100g protein, respectively, including methionine +cysteine, isoleucine, phenylalanine, threonine, valine and lysine with slightly differences between JR and TN. Data showed that, phenylalanine + tyrosine content was higher in JR than in TN. However, the overall quality of protein in the JR compromised by its high phenylalanine+ tyrosine content 6.13 % of the total essential AA. Tryptophan was the least concentrated, in JR with values of 0.82 g/100g protein.

Table 2. Amino acids (AA) content (g/100g protein) of JR and TN

AA composition	WHO* ideal AA(g/100) protein)	JR		TN	
		AA	% WHO	AA	% WHO
Essential AA					
Isoleucine	2.8	1.84	65.7	1.24	44.29
Leucine	6.6	2.34	35.5	2.37	35.91
Lysine	5.8	2.96	51.0	2.47	42.59
Methionine+Cysetine	2.5	1.22	48.8	1.13	45.20
Phenylalanine+Tyrosine	6.3	6.13	97.3	1.86	29.52
Phenylalnine	-	2.13	-	0.99	-
Tyrosine	-	4.00	-	0.87	-
Threonine	3.4	2.24	65.9	1.86	54.71
Tryptophan	1.1	0.82	74.5	1.24	112.73
Valine	3.5	2.34	66.9	2.16	61.71
Total essential AA	32.0	19.89	62.16	14.33	44.78
Non-essential AA					
Aspartic		11.68		13.12	
Glutamic		18.61		17.50	
Serine		3.47		5.83	
Proline		12.96		5.47	
Glycine		4.01		6.93	
Alanine		5.11		8.02	
Histidine		3.10		2.92	
Arginine		21.17		25.88	
Total non-essential AA		80.11		85.67	
P-PER		0.55		0.52	

P-PER: Predicted protein efficiency ratio. *WHO (1985) WHO/FAO Report.

However, JR contained the second predominant AA of lysine; leucine follows by valine as 2.16, and similar ratio of leucine and valine 2.34 g/100g from total essential AA. In Table 2, TN contained an abundant AA ratio of lysine (2.47g/100g), follows by leucine (2.37 g/100g) and valine (2.16 g/100g). These results are lowering than obtained by Temple *et al.* (1989) who reported that chufa contain lysine (4.9 g/ g/100g protein), leucine (2.9 g/100g protein) and valine (2.5 g/100 g protein). It is interesting to note that, the phenylalanine + tyrosine content of the JR was provide 97.3% of the

WHO ideal protein standard. Proportion of tryptophan and follows by threonine and valine in JR provide 74.5 and about of 66.0 % for the ideal standard protein of WHO. Total essential AA in TN (14.33 g/100g protein) which in lower than in JR (19.89g/100g protein). TN amino acid pattern provide about 11.3% of tryptophan was higher than WHO standard protein for children (Table 2). Compared to standard percentage amino acid in WHO profile , both of JR and TN were lacked in leucine and aromatic amino acids (phenylalanine+ tyrosine) , respectively.

Concerning non-essential AA content of JR and TN the results revealed that, the major abundant non-essential AA in JR were arginine acid, glutamic acid, proline and aspartic with values 21.17, 18.61, 12.96 and 11.68 g/100g protein, respectively. TN contain high amount of arginine, which liberates insulin hormone and provides some digestive enzymes like catalase, lipase, and amylase, it could recommended for those who have problems with digestion, flatulence and diarrhea, also TN milk is a suitable drink for celiac patients and for the lactose-intolerant (Adejuyitan, 2011). Total non-essential AA for JA and TN accounted

80.11 and 85.67 g/100g protein, respectively. The Predicted Protein Efficiency Ratio (P-PER) is one of the quality parameters used for protein evaluation (FAO/WHO, 1991). The P-PER of JR and TN was 0.55 and 0.52, respectively. This study showed that JR and TN nutritionally useful quantities of most of the essential amino acids and can serve as food supplements.

Fatty acids composition in JR and TN:

Fig 1. illustrated fatty acids composition of crude lipid fraction from JR and TN. The results revealed that, JR fatty acid profile is predominant in linoleic acid, c18:2n6 (55.34 %) and follows by palmitic acid, C16:0 (29.04%) and linolenic acid, C18:3n3 (9.6%), these account more than 94% of total fatty acid. Oleic acid is the major fatty acid in TN ratio about 70% follows by palmitic acid, C16:0 and Linoleic acid, c18:2n6 (14.5 % and 8.8%, respectively). Similar result by Muhammad *et al*. (2011) who found that, TN oil is predominantly consists of oleic acid with values ranging from 65.5 to 76.1%. Dubois *et al*. (2007) reported that , the major fatty acids in TN oil are 14:0 (0.2%), 18:0 (3.2%), 20:0 (0.4%), 16:1 n −7 (0.3%), 18:1 n−9 (72.6%), 18:2 n−6 (8.9%), and 18:3 n−3 (0.4%). Moreover, TN oil has a monounsaturated profile (>60% monounsaturated fatty acids (MUFA)), similar to fatty acid profile of olive oil, hazelnut, macadamia nut, avocado, and apricot kernel oils (Dubois *et al*. 2007). Tigernuts reported as a helping agent in prevention heart attack and thrombosis by enhancing blood circulation, reducing low density lipoprotein (LDL-C) and increasing high density lipoprotein (HDL-C) (Belewu and Abodunrin, 2006). Daily consumption of TN has been shown to be effective in weight loss and improvement the metabolic disorders among obese diabetic patients (Salwa *et al*. 2010).

Polysaccharides content of JR and TN:

As shown in Figure (2) the polysaccharide content in JR and TN were defiantly different between each of them. Inulin was the most abundant in JR (75.16 g), follows by sucrose (4.8 g) and glucose (1.5 g). This result is also obtained by Franck (2000) who indicated that, JR is contains 95% inulin on dry matter. Fructose was the most abundant sugar in TN (13.5 g), follow by

glucose and inulin (10.0 and 4.8 g, respectively), therefore, TN is suitable for diabetes. Inulin and fructose considered as bioactive ingredients, which might be surprise as relevant to modify and improve the technical production of fermented milk and yoghurts. Inulin at ratio 2-4% is increase the firmness of fermented milk by *Streptococcus* and inoculated with *Bifidobacterium lactis* (Pinheiro *et al*. 2009). Gibson *et al*. (2004) reported that, the polysaccharide inulin is a soluble dietary fiber, which is not degraded by enzymes in the human digestive system, but fermented selectively by beneficial bacteria in the gut. Inulin and its degradation products are capable of stimulating and/or activating health-promoting bacterial growth in the colon. Moreover, inulin increases blood glucose level less than starch, and it is therefore suited as a constituent in an anti-diabetic diet (Rumessen *et al*., 1990).

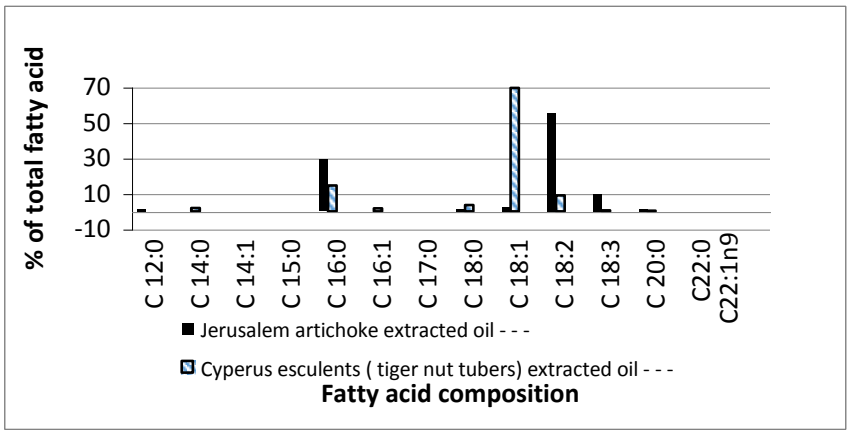

Fig 1. Fatty acids composition of JR and TN

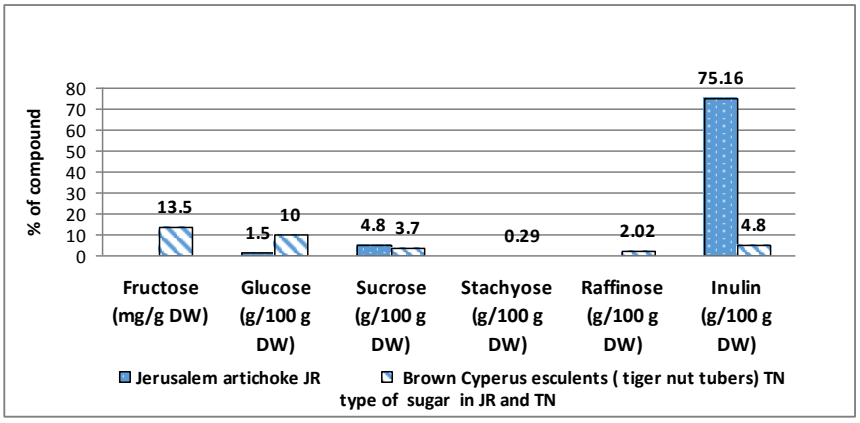

Fig 2. Polysaccharides content in Jerusalem artichoke and tiger nut tubers

Vitamins content in JR and TN:

The most abundant vitamin compound in JR and TN were vit E and vit B1 and B2, these vitamins were the main task in human nutrition and antioxidant role in biological systems (Table 3). In terms of their nutrient content, the TN seeds compare richen in vitamin compare with those of JR; except for Vit B2, both plants seem capable of satisfying less than 6% of an adult's requirement for the various nutrients listed in Table (3). The TN provided an adequate quantity of Vitamins E and B1 about 54 and 21%, respectively versus than RDV recommendation. A quite similar vitamin content in JR when compared to TN, vitamin E and Vit B1 accounted 47.33 and 13.27 as % of RDV, respectively. The obtained results were agree with Belewu *et al*. (2007).

Table 3. Vitamins content in JA and TN (100 g dry base)

Vitamins	RDV*	*JR*		TN	
		Contents**	% of RDV	Contents **	% of RDV
Vit B1 (mg)	1.50	0.199 ±0.48	13.27	0.31±0.35	20.67
Vit B2 (mg)	1.70	0.089±0.16	5.24	0.10±0.63	5.88
Vit E (mg)	15	7.1±0.19	47.33	8.0±0.13	53.33
Vit C (mg)	90	1.5±0.62	1.67	5.4±0.02	6.00

***Relative daily value (RDV); from the Food and Nutrition Board (2002) ; ** Values represent the mean ±SD of triplicate measurements**

Minerals content in JR and TN:

Minerals content in JR and TN illustrated in (Table 4), JR contained a relatively large amount of micronutrient and macro nutrient elements than in TN such as Zn, Mo, P and Cu accounting (17.17, 11.92, 370 and 5.35 mg/kg dry weight, respectively). However, TN was containing a considerable amount of sulfur, Mn, Ca, Mg and Fe (1369, 11.39, 150, 132.3 and 131.5 mg/kg on dry base, respectively). These minerals are important for human. 100 g of JR provide a sufficient amount more than quarter ratio of copper USDA recommend. Both of JR and TN are good sources of Fe, Zn, and Mg, while TN is only contain a higher ratio of Cu. These elements are very important in human malnutrition and diabetic diseases (Edem *et al*. 2009). Iron is necessary for the prevention of anemia, and zinc necessary for nucleic acid metabolism, protein synthesis and cell growth (Igoe 1989). Molybdenum is an essential element in human nutrition, its enhanced stress response in human body which exposure to xenobiotic compounds and involves detoxification of these compounds (Luo *et al*. 1983).

Table 4. Mineral contents of JR and TN.

	Minerals mg/kg								
	Ca	P	Mg	Mo	Fe	Zn	Cu	Mn	S
JR	120	370	1127	11.92	117.2	17.17	5.35	10.84	1250
TN	150	330	1323	9.75	131.5	12.11	ND	11.39	1369
RDV-(mg)	1000	1000	400	75	18	15	2.0	2.0	-

***Relative daily value (RDV); from the Food and Nutrition Board (2002)**

GC-MS analysis in JR and TN:

Data from the analysis of JR and TN volatile components are illustrated in Table (5). It showed that, 20 compounds in JR, and 18 compounds in TN were tentatively identified. Most of these methanolic extract from two plants are bosses as bioactive compounds and antimicrobial agents. The largest portion of GC-MS profile of JR was propionic acid, nonal ester (29.605%), followed by D-allothreonine (11.84%) and Acetic acid, ethoxyhydroxy-, ethyl ester (10.526%). D-allothreonine is one of essential amino acid, which maintains phospholipid metabolism and physiology in liver cells (Kathayat *et al*. 1997). The largest portion of GC-MS profile in TN was l-(+)-Ascorbic acid 2,6-dihexadecanoate (40.773%), followed by 2-Butenedioic acid (E)-, bis(2-ethylhexyl ester (13.305%) and palmitic acid (10.730%) of total identified GC MS compound. Moreover, TN contains hydrocarbon compounds such as B-Cymene, D-Limonene and Psi-limonene in ranged between 1.5-2.57% from total GC-Ms profile. This result is agree with Kubmarawa *et al*. (2005) who reported that, TN contained high amounts were ρ-cymene (1.3–2.8%), limonene (1.3–2.8%), myrcene (1.7–1.8 %) and sabinene (1.0–6.9 %). B-Cymene, which has a biological role in antimicrobial activity, as well as two aromatic alcohols of O-thymol and menthol were identified in TN.

Huisman *et al*. (2004) reported that, JR contained many important alcoholic compounds such (-)-isopulegol, 2-p-cymenol, thymol acetate and geranyl isovalerate, these alcoholic material exhibited antioxidant properties. Caryophyllene compound such as ɣ-Gurjunene has been identified in JR at ratio 3.947% of total identified GC-MS compounds. Several biological activities attributed to *β*-caryophyllene, such as anti-inflammatory, antibiotic, antioxidant, anticarcinogenic and local anaesthetic activities. This result agree with Legault and Pichette (2007). In addition, caryophyllene oxide recognized as stabilizer in foodstuff, drugs and cosmetics and has shown growth inhibiting activity against dermatophytes (Yang *et al*. 1999). It has also shown growth inhibitory effects on *Staphylococcus aureus* (Katsuyama *et al*. 2005). Thymol (phenolic monoterpenes) was identified in both plant of JR and TN. It has relatively strong antimicrobial activities (Burt 2004). It was synergistically active against *E. coli* strains acting by thymol disintegrated the outer membrane of *E. coli* (Lambert *et al*. 2001).

Fatty acid (FA) composition of bio-yoghourt:

Table (6) represents the overall FA composition in bio-yoghourt fortified with JR or TN flour, 17 FA detected, comprised of both saturated and unsaturated FA. Long chain FA profile of control bio-yoghourt shows Palmitic (C16:0), oleic acid (C18:1), myristic (C16:0) and stearic acids (C18:0) being relatively high. Relative proportion of different FA is underwent changes during processing. Fortified bio-yoghurt with JR induced changes than control yoghurt, where it decreased oleic acid (8.44), increased low molecular FA as caproic (C6:0) and caprileic acid, (C8:0), as well as increased linoleic acid (C18:2n6) up to 18.17%, which is 6 times more than the amount in the control sample.

Table 5. GC-MS components of JR and TN methanol extract.

RT	Compound name in JR	Peak area %	Compound name in TN	Peak area %
3.22	Propionic acid , nonal ester	29.605		
3.23			2-Butenedioic acid (E)-, bis(2-ethylhexyl ester	13.305
3.31	Butanoic acid , 2,4-diamino	4.934		
3.71	Acetic acid, ethoxyhydroxy-, ethyl ester	10.526		
3.81	D-allothreonine	11.842		
4.75			O-thymol	1.073
6.36			Acetic acid, 2,2-{oxybis(2,1-ethyl)ester	1.073
6.81	Phenol, 4-(2-aminopropyl)	2.303		
7.04			B-Cymene	2.146
7.09			D-Limonene	2.575
8.15	(-)- ISOPULEGOl	2.303		
8.16			Psi-limonene	1.502
9.31			Menthol, (±)-	2.146
9.41			Cis-B-Terpinenol	0.858
9.67			Benzoic acid, 2-hydroxy-, hydrazide	3.863
10.70	Methoxyacetic acid , 2-pentadecyl ester	1.645		
10.22	2-P-CYMENOL	2.303		
10.92			Anethole	0.773
11.1			Carvacryl acetate	6.438
11.12			5-Isopropenyl-2-methyl-2-cyclohexen-1-one	5.579
11.3	Thymol acetate	2.467		
12.25	Geranyl isovalerate	1.974		
12.92	Methoxyacetic acid, 3-tetradecyl ester	1.974		
13.47	Eicosane,10-Methyl	1.974		
13.73	ɣ-Gurjunene	3.947		
13.99			Geranyl isovalerate	0.773
14.73			Butyric acid, 4-pentadecyl ester	1.073
14.74	Butyric acid, 4-pentadecyl ester	4.934		
15.9	TETRADECANE, 2,6,10-TRIMETHYL	1.974		
16.36	TRICOSANE	3.947		
16.47			Stearic acid	1.717
18.13	OCTADECANE	3.618		
18.13			Hexadecanoic acid, methyl ester	3.433
18.6	Nonahexacontanoic acid	2.303		
18.71	Phthalic acid, isobutyl octadecyl ester	2.632		
18.74			I-(+)-Ascorbic acid 2,6-dihexadecanoate	40.773
18.92	Hexadecanoic acid, methyl ester	2.632		
18.93			Palmitic acid	10.730

Linoleic acids (CLA) has attributed as anticarcinogenic properties, as well as antiatherogenic effects, and also known as rumenic acid (Masso-

Welch *et al*. 2004). Fortified bio-yoghurt with TN showed higher oleic acid and lower C18:2n6 than found in JR bio-yoghurt. Fortified bio-yoghurt with TN increased oleic acid from 22.35% in control to 35.70%, as well as increased CLA up to 7.10%, while decreased Stearic acid than control bio-yoghurt. Low molecular FA is lower in TN bio-yoghurt than in control and JR bio-yoghurt. These FA are responsible to improve aroma of processed yoghurt. It seems that individual fatty acids followed different pattern of changes during processing or fermentation. Therefore, it assumed that, fortification bio-yoghurt containing probiotic bacteria with JR or TN led to improve growth of probiotic bacteria and produce important CLA, which has anticancer and anti hypercholestrolemic activities.

Table 6. Fatty acid composition of bio-yoghourt fortified with JR or TN flour.

Fatty acids	Control	Fortified with JR	Fortified with TN
Caproic Acid, C6:0	2.02	3.29	2.25
Caprilic Acid, C8:0	1.19	2.28	0.25
Capric Acid, C10:0	4.28	3.19	2.48
Lauric Acid, C12:0	4.16	3.22	1.17
Myristic Acid, C14:0	12.38	10.24	9.28
Myristoleic Acid, C14:1	1.16	2.25	2.28
Pentadecanoic Acid, C15:0	2.18	0.99	0.72
Palmitic Acid, C16:0	27.87	25.23	28.59
Palmitoleic Acid, C16:1	1.95	0.50	0.33
Heptadecanoic Acid, C17:0	1.41	0.23	0.28
Stearic Acid, C18:0	10.28	12.15	6.24
Oleic Acid, C18:1n9c	22.35	8.44	35.70
Linoleic Acid, C18:2n6	3.62	18.17	7.12
Linolenic Acid, C18:3n3	3.84	8.69	2.78
Arachidic Acid, C20:0	0.22	0.59	0.23
Behenic Acid, C22:0	0.87	0.54	0.16
Eurcic Acid C22:1n9	0.22	-	0.14

Texture profile of bio-yoghurt fortified with JR and TN:

The instrumental texture profile of bio- yoghurt fortified with 10% of JR or TN have shown in Table (7). There was an increase in all texture parameters analyzed. Firmness of fortified bio-yoghurt was increased from 5.55 for control to 8.21and 9.22 for JR and TN, respectively. There is no variation between fortified bio-yoghurt with JA or TN, whereas, there were increase in cohesiveness compare to control bio-yoghurt. Gumminess, chewiness and springiness of different bio-yoghurts 2.16, 1.08 and 0.33 to 2.22, 1.29 and 0.48, respectively showed differences between JR and TN bio-yoghurt. The stability of texture profile was desirable to maintain physical-chemical and sensory properties after fermentation and storage period. The increase in firmness may be related to dietary fiber absorbing more moisture because of

its higher water-holding capacity (Hashim *et al*. 2009), and oil-holding capacity, emulsification and/or gel formation. (Elleuch *et al*. 2011). It has been suggested that inulin is a water-structuring agent and it may form a complex with protein aggregates in yoghurt, which could explain the increase in firmness in these products (Kip *et al*., 2006). Similar to Srisuvor *et al*. (2013) and Oliveira *et al*. (2011) inulin addition to co-cultures and cocktail enhanced products firmness, either after 1 day or 7 days of cold storage, likely due to the increase in microbial growth induced by metabolic interactions among lactic acid bacteria and partial inulin metabolization. Moreover, Tamime (2005) found that the higher microbial growth is one of the causes of a firmness increase in yoghurt.

Table 7. Texture profile analysis of bio-yoghurt fortified with JR or TN (10%) flour

Texture profile	Control	Fortified with JR	Fortified with TN
Firmness (N)	5.55±0.29	8.21±1.35	9.22±0.99
Cohesiveness	0.25 ±0.22	0.78±0.11	0.79±0.18
Gumminess (g)	1.28±0.13	2.16±1.47	2.22±1.14
Chewiness (g×mm)	0.39±0.04	1.08±1.31	1.29±0.90
Springiness (mm)	0.24±0.12	0.33±0.09	0.48±0.78

Table 8. Organoleptic evaluation for bio yogurts fortified with 10% JR or TN

Taste parameter	Control	Fortified with *biofedobacterium* and JR	Fortified with *biofedobacterium* and TN
Sourness	6.3± 1.9	6.5± 2.2	5.3± 1.2
Bitterness	4.2± 1.2	3.1± 0.7	2.2± 1.6
Sweetness	2.6± 1.6	4.3± 1.5	5.7± 1.0
Aroma	6.3± 0.8	4.2± 1.7	5.8± 2.1
Overall acceptability	6.7± 0.2	5.3± 1.8	7.1± 1.7

Organoleptic evaluation for bio- yogurts:

Results from Table (8) showed organoleptic evaluation of bio-yoghurt fortified with 10% of JR or TN. Sourness was decrease in fortified TN. The TN bio-yoghourt was lower in brightness than control and JR bio-yoghurt. Characteristics of color of fortified TN were naturally brown, which affect the overall color and brightness. Increasing of sweetness in TN bio-yoghurts attributes to the level of sweeteners of fructose and glucose content in TN compared to control and JR yoghurts. Bio-yoghurt fortified with JR has impassive in aroma than control and TN yoghurt. There was a defiantly higher overall acceptability in TN than control and JR bio-yoghurt. These contribute to that TN composite was contain a higher ratio of natural

sweeteners with lowering of sourness. The addition of each prebiotic could improve

physical and sensory properties of the yoghurt, by stimulating growth of probiotic bacteria (Srisuvor *et al*. 2013). Moreover, Decourcelle *et al*. (2004) mentioned that conducted those probiotic yoghourts with high level of TN characterized with softness and sweet flavor.

Rheological properties of dough:

The results obtained from alveographic measurement of wheat flour (WF) substituted with JR and TN summarized in Table (9). Substitute WF with 5, 10 and 15% of JR or TN evaluated as weaker with lower tenacity and baking strength parameters. Wheat flour is able to form cohesive dough having viscoelastic properties and possessing the ability to retain gas, prepared from these flours at different ratio of substitutions did not have optimal viscoelastic behavior, as described by the lower in Configuration rate (P/L) percentage. These results are presented the dough rheological behavior during fermentation and baking. Therefore, the Alveograph device allows the measurement of gluten deformation. Its mode of deformation is similar to the extension that takes place during fermentation and oven rise.

The baking strength representing the energy necessary to inflate the dough bubble to the point of rupture ranged from 160 in the control wheat flour (100%) to 36.0 in the ratio 15 % TN substitution in WF. Such a weakening tendency of the dough for blends JR or TN with flours substitution with low tenacity values is characteristics to the presence of a low molecular weight dextrin produced by hydrolyses of damaged wheat flour starch during fermentation assay. Generally , elastic modulus , extendibility , index of swelling , baking strength , configuration rate were lower than present in control 100% wheat flour. Wang *et al*. (2002) reported a decrease in dough elasticity, determined by a farinograph test, upon addition of 3% chicory inulin (Collar *et al.* 2007). The addition of 10 and 15% of JR or TN has produced worst quality of dough elasticity and extensibility. This result is worth mentioning that addition ratio of JR and TN at 5% have a little closest of Alveograph parameters with 100% wheat flour dough.

Microbiological evaluation:

Effect of *JR* and *TN* on some probiotic bacteria in *vitro*:

The results recorded in Table (10) clearly showed that JR at concentration 10% encouraged growth of *L. plantarum* ; *L. curvatus* and *B. subtilis* in enrichment broth medium and following by increasing counts of *L. plantarum* from 4×10^{13} to 3×10^{17} , *L. curvatus* from 4×10^{13} to 4×10^{17} and *B. subtilis* from 4×10^{11} to 4×10^{16} cfu/ml, while TN at concentration 10 % increased counts of *L. plantarum* from 4×10^{13} to 8×10^{16} , *L. curvatus* from 4×10^{13} to 4×10^{17} and *B. subtilis* from 4×10^{11} to 13×10^{16} cfu/ml in enrichment broth medium. The concentration 10 % of JR or TN represented the optimum concentration for enhancing the growth of the three-probiotic strains and led to decreasing pathogenic bacteria by reduction acidity. Fortified yoghurt by JR or TN, improved carbohydrate content and prebiotic inulin, which contributed to increase activity of *Lactobacillus plantarum* and

Table 9. Rheological properties of wheat flour (WF) dough supplemented with different concentration of JR or TN

Flour alveograph properties	WF	JA			TN		
	100%	5%	10%	15%	5%	10%	15%
Tenacity (P) mm H_2O	85.7	76.0	54.0	38.00	89.0	44.00	46.00
Extensibility (L) mm	53.1	46.0	41.0	29.00	29.0	32.0	18.0
Index of swelling (G)	17.2	15.1	14.3	12.0	12.0	12.6	9.4
Baking strength (W)10E-4 J	160.8	151.0	94.00	46.0	113.0	57.00	36.0
Configuration rate (P/L) %	1.77	1.65	1.32	1.31	3.07	1.38	2.56
Elasticity index (Ie) %	75.6	60.00	51.4	45.20	55.6	43.8	36.15

Table 10. Effect of JR and TN on some probiotic bacteria counts *in vitro*

	Treatments*			
without	**JR**		**TN**	
	5%	**10%**	**5%**	**10%**
L. plantarum 7×10^{13}cfu/ml	2×10^{15}	3×10^{17}	3×10^{16}	8×10^{16}
L. curvatus 9×10^{13}cfu/ml	3×10^{16}	4×10^{17}	5×10^{16}	4×10^{17}
B. subtilis 9×10^{11}cfu/ml	5×10^{14}	4×10^{16}	10×10^{14}	13×10^{16}

***The used inoculum of *L. plantarum* was 4×10^{13} cfu/ml; *L. curvatus* was 4×10^{13} cfu/ml and *B. subtilis* was 4×10^{11} cfu/ml**

Lactobacillus curvatus than control yoghurt. This also recorded by Buriti *et al*. (2010a) and Komatsu *et al*. (2013).

Effect of JR and TN on growth and survival of *Staph aureus* in *vitro*:

The obtained results in Table (11) showed that, JR and TN at concentration 10.0 % resulted in decrease of *Staph aureus* counts from 7×10^{13} to 6×10^{7} and 5×10^{6} cfu/ml, respectively in *vitro* and to 9×10^{4} and 7×10^{3} cfu/g, respectively in yoghurt. On the other hand, non-fortified yoghurt samples showed reduced *Staph aureus* counts from 7×10^{13} to 2×10^{9} cfu/g, these results in agreement with the studies of Mattila –Sandholm *et al*. (2002) and Dimitroglou *et al*. (2011) which recorded that, probiotics prevent and control of pathogenic microorganisms as an alternative against traditional disease control such as chemotherapeutic agents or vaccines (Dimitroglou *et al.* 2011). The addition of each prebiotic could improve physical and sensory properties of the yoghurt, by stimulating growth probiotic bacteria (Srisuvor *et al.* 2013). Inulin addition to co-cultures and cocktail enhanced products firmness, either after 1 day or 7 days of cold storage, likely due to the

increase in microbial growth induced by metabolic interactions among lactic acid bacteria and partial inulin metabolization (Oliveira *et al.* 2011*).* Stimulatory effect of inulin on the growth of *bifidobacteria* and lactobacilli (Akalin *et al.* 2004), has recently been described to greater release in yoghurt of additional nutrients (Makras et al. 2005). Moreover, probiotics can inhibit pathogen multiplication , where low numbers of *Lactobacillus delbrueckii* would help the survival of probiotic organisms due to reduced risks of post acidification by *Lactobacillus delbrueckii* (Shah 1995). In addition, inulin powder improved *Lactobacillus casei* growth during fermentation and their survival during storage time (Aryana and McGrew 2007), higher counts of probiotic bacteria in yoghurts with medium and long Chain inulin than those with oligofructose at the end of storage (Lankaputhra *et al*. 1996).

Table (11): Effect of JR and TN on growth and survival of *Staph aureus* **in vitro*

Treatments*				
without	**JR**		**TN**	
	5.0%	**10.0%**	**5.0%**	**10.0%**
In vitro 9×10^{13}cfu/ml	3×10^{9}	6×10^{7}	4×10^{7}	5×10^{6}
Yoghurt 2×10^{9}cfu/g	4×10^{6}	9×10^{4}	3×10^{4}	7×10^{3}

***The used inoculum for *Staph aureus* was 7×10^{13} cfu/ml**

The supplementation inulin from Jerusalem artichoke resulted in greater growth rates of *Lactobacillus casei* than *Bifidobacterium bifidum* and *Lactobacillus acidophilus* during cold storage of yoghurt (Paseephol and Sherkat, 2009). The mechanism by which inulin improve the viability of the probiotic organisms during cold storage is still unclear, while the two possible mechanisms proposed so far state that inulin's provide additional nutrients for promoting culture growth (Makras *et al*. 2005), and that they protect probiotic cells from acid injury (Desai *et al*. 2004). The addition of inulin reduced the fermentation time by about 10.0% as an average, thus confirming its prebiotic effect already evidenced for both *biofidobacteria* and *lactobacilli* by (Donkor *et al.* 2007). The higher microbial growth is one of the causes of a firmness increase in yoghurt (Tamime, 2005), while some other publications by Ozer *et al.* (2005) reported , that inulin didn't support the growth and survival of *L. acidophilus* in fermented bovine milk and acidophilus-bifidus yoghurts.

CONCLUSION

The results from this study revealed that TN oil contains FA similar of olive oil. Adding JR and TN in bio-yoghurts improve nutritional values, probiotic bacteria and the stability of texture profile, which is desirable to maintain physical-chemical and sensory properties after fermentation and storage period. In addition, substitution of JR and TN in white flour WF improve nutritional quality and lowering gluten composite, which is suitable for Celiac disease patients. 10.0% supplementation level of JR and TN

resulted in greater rates of probiotic bacteria growth in *vitro*, and showed stronger antimicrobial activity against *Staph aureus* in *vitro* and processed yoghurt. Overall results suggest that JR and TN are a potential functional food ingredient that may be used in food applications.

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تأثير إستخدام المصادر السكرية لنباتي الطرطوفة وحب العزيز كمواد بريبيوتيك في بعض الأغذية الوظيفية من الزبادي والعجائن على نمو البكتريا المرضية وبكتريا البروبيوتيك بدراسة ميكروبيولوجية وتغذوية

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المركز الاقليمي للأغذية والأعلاف – مركز البحوث الزراعية

لتحسين إنتاج أغذية محتوية علي البروبيوتيك تطرقت هذة الدراسة إلي امكانية إستخدام المصادر السكرية لكلا من نباتي الطرطوفة وحب العزيز كمصادر أولية للبريبيوتيك وتقييم أداء إنتاجها ونموها في الأغذية المتخمرة كالزبادي والعجائن ومدي تأثيرها علي حفظ تلك المنتجات من خلال تقييم جودتها المختلفة وكذلك مستوي البكتريا الممرضة في تلك الأغذية ومدي إيقاف وتثبيط بكتريا الإستاف الممرضة . ويعد الأنيولين المتوافر في الطرطوفة من أهم المواد التي لها دور وظيفي كبريبيوتيك مسببة لنشاط البكتريا المفيدة صحيا وتغذويا.وكذلك تم التطرق إلي كل مكونات تلك النباتات كيماويا وتحديد المغذيات الكبري والصغري من مطحوتها بعد معاملة حب العزيز بالنقع والتجفيف.

تم تقدير محتواهما من الأحماض الأمينية والأحماض الدهنية والتحليل الكروماتوجرافي الكتلي وتركيب السكريات المختلفة واستخدم إضافات للعجائن بنسب مختلفة من كلا الدرنات بعد طحنها في إنتاج عجائن تتميز بانخفاض مستوي الجلوتين الذي يناسب بعض المرضي سواء مرضي السكر أو مرضي السيلك . وقد تم التحاليل الميكروبيولوجية علي البكتريا سواء المنشطة من البروبيوتيك او الممرضة والمسببة لفساد الأغذية من بكتريا الإستاف في الزبادي المدعم بكلا المصادر من الدرنات محل الدراسة . وقد اظهرت الدراسة إلي إمكانية إستخدام تلك الدرنات في إيجاد أغذية وظيفية لبعض المرضي والحالات الغذائية فضلا عن إيجاد مصدر إقتصادي لتلك الاغذية الوظيفية.