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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) EI - Reffaei, W. H. M.; A. F. Abdel - Salam; A. M.M. EI-Kharamany and Eman M. Ragheeb Regional Center for Food and Feed (RCFF), Agric. Res. Center, Giza, Egypt. EFFECT OF Jerusalem artichoke AND Tigernut AS SUGAR SOURCES ON PROBIOTIC GROWTH IN SOME FUNCTIONAL FOOD (BIO - Salam, AL SUGAR SOURCES ON PROBIOTIC GROWTH IN SOME FUNCTIONAL FOOD (BIO-YOGHUW HEAT DOUGH).

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 Jerusalemartichoke (JR) and Cyprus esculents (Tigernut or Chufa) (TN) as prebiotics source, in-to improve grow th 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 w heat 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 show ed that, inulin was the most abundant poly saccharides in JR while TN is 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 grow th 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.

Key-words:-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*_n 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*_n 2002). The most important prebiotics are fructans (inulin and oligofructoses), glucans and mannans, which are soluble and fermentable fibers (Gibson *et al*_n 2004).

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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 <u>Oliveria 2011). Inulin is among the most famous prebiotic compounds. Also</u> 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 longchain inulin-type fructans significantly increases calcium absorption and enhances bone mineralization during pubertal growth. Effects of dietary factors on calcium absorption maybe 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.

There is a strong relationship between food and medicine so that in many cases, the food use is not separable from its medicinal action, a specific association between foods/nutrients and health functions, consumers clearly believe in the concept of functional nutrition (Pieroni, 2003). Prebiotic foods including dairy products defined as "foods containing live microorganisms, which believed to enhance health by improving the balance of micro-flora in the gut" (Tamime et al., 2005). The microorganisms that most commonly used as probiotics belong to the heterogeneous group of lactic acid bacteria (Lactobacillus, Enterococcus...etc) and to the genus Bifidobacterium (FAO/WHO, 2001). Foods containing such bacteria fall within the category of functional foods, which described as foods claimed to have a positive effect on health. Probiotics are able to be effective in the treatment of several intestinal disorders and have an impact on the immune system (Foligne et al, 2007). Lactobacillus casei Shirota fermented milk enhances cytotoxic activity of natural killer cells (Takeda et al., 2006), and lactic acid bacteria play a beneficial role in the ecosystem of the human intestinal tract (Jia et al. 2008). Furthermore, consumption of probiotic products is helpful in maintaining good health, restoring body vigor and skirmishing intestinal and other diseases. Probiotic is considered as a functional ingredient because it is not absorbed in the small intestine and exerts a prebiotic effect on the intestinal habitat, which causes normalization of stool frequency (increases

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J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 6 (8), August, 2015

the number of bacteria and/or activity of the number of *bilidobacteria* and lactic acid bacteria in the human gut) (**Rodrigues** *et al.*, **2011**). Thus, probiotic bacteria offer new dietary alternatives for the management of such conditions through stabilization of intestinal microflora, promotion of colonization resistance, regulation of the immune response and preservation of intestinal integrity (**Farnworth, 2008**).

Fermented dairy products have been the most utilized food matrix for probiotic intake. Several studies have related the promising health benefits of consuming culture containing milks. Lactobacilli (Lactobacillus and Bifidobacterium spp.) (LAB) are important inhabitants of the intestinal tract of men and animals and are involved in a number of potential health beneficial roles viz., immune modulation, pathogen exclusion, production of antimicrobial substances, anticarcinogenic and cholesterol lowering activities (Farnworth, 2008). Furthermore, LAB strains synthesize short chain fatty acids, vitamins, and exopolysaccharides (EPS) that employed in the manufacturing of fermented milk to improve its texture and viscosity (Ruas-Madiedo et al., 2002). Studies have proven the successful incorporation of probiotics into yogurt as well as other fermented milk products; such as, soy yogurt (Farnworth et al. 2007 and Hekmat et al., 2009). The ability of probiotic bacteria (Lactobacillus acidophilus La5 and Bifidobacterium animalis Bb12), to produce conjugated linoleic acid (CLA) in association with Streptococcus thermophilus and L.b. bulgaricus during milk fermentation has been evaluated in this study by (Manzo et al., 2015), CLA contents of 10 commercial fermented milk products were determined. The highest content has observed in fermented milk containing only Str. thermophiles and Lb. bulgaricus.

Chufa, Cyperus esulentus, root stock snack or earth almond, commonly called "Tigernut, (TN) belongs to the genus Cyperaceous; it 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 Southem Europe. It is a perennial tuber commonly found in Egypt and Spain, as well as, in some other countries around the Mediterranean region (Coskuner et al., 2002). The spherical underground tubers are edible and have a sweet nutty flavor, it consume fresh, dried or roasted, and recorded as having medicinal properties (Negbi, 1992 and Barminas et al. 2001). The major components of this tuber are complex carbohydrates. It is comprise fructos ylfructose linked compounds such as inulin, which are associated with several physiologic actions in the small and large intestine, thus showing several important metabolic implications for health (Roberfroid, 2001). Its protein contentis rich in arginine, which liberates hormones that produce insulin; thus being suitable for diabetics (Adejuyitan, 2011), furthermore minerals (phosphorus and potassium), vitamins (Vit E and C), steroids and triterpenes thus making this tuber suitable for diabetics. It has reported to be a health food, since its consumption can prevent heart disease, thrombosis, activate blood circulation and reduce the risk of colon cancer (Frega et al., 1984 and Arafat et al., 2009 and Chukwuma et al., 2010). Tigernut's oil known for its high-unsaturated fatty acid contents and thus can be useful for human

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nutrition in replacing conventional sources of oil (Makai and Balantincz, 2002). This tuber commonly used for the production of milk, nonalcoholic beverages, and used as food additives and spices (Umeri and Enebeli, 1996).

Prebiotics are non-digestible components of functional food that stimulate the proliferation and activity of bacterial population desirable in the colon and inhibit pathogen multiplication, hence beneficially acting on the host (Roberfroid, 2000 and Marttila 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). It not digested by aamylase or other hydrolases in the upper section of the intestinal tract (Carabin and Flamm, 1999 and Piad et al., 2006). In human nutrition intervention trials, inulin appeared to be more effective than oligofructose in reducing triglyceridemia (Reimer and Russell, 2008). In young adolescents, daily consumption of a combination of prebiotic short- and long-chain inulintype fructans significantly increases calcium absorption and enhances bone mineralization during pubertal growth (Abrams et al., 2005). Oligosaccharides suppress potentially deleterious bacteria among the gastrointestinal microbiota (Kajawara etal., 2002). Increase in the number of beneficial Bifidobacterium spp. in the intestinal microbiota has been the mean focus of prebiotic addition (Akalin et al., 2004 and Gibson et al., 2004). Oliveria (2011) demonstrated that, 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 (Akin et al., 2007 and and Cruz et al., 2010). Among other plants rich inulins is Jerusalem artichoke (Helianthus tuberosus L.) (JA), its tuber accumulate similar levels of inulin (10-20 % of fresh tuber) as chicory roots (Frank, 2000) and could be cultivated at a low cost with low input of fertilizers on any type of soil and cool climatic conditions (Parameswaran, 1994). However, the use of JA tubers for inulin extraction is less well known as they are commonly eaten as vegetable. When inulin added to food in low concentrations the rheological properties and sensory quality of the product not be affected strongly due to the neutral or slightly sweet taste and the limited effect on the viscosity of this ingredient (Kalyani et al., 2010).

Akalin et al. (2007) study conducted to stimulate the growth of probiotic bacteria during yoghurt fermentation and to improve their survival until the use - by-date, by supplementing yoghurt milk with growth factors such as vitamin enriched protein hydrolsate, amino nitrogen and whey protein concentrate. Co-culturing with proteolytic yoghurt bacteria i.e. *Lactobacillus delbrueckii subsp. Bulgaricus* (LB) and *Stroptoccus thermophilus* (ST) also enhances the growth and the viability of probiotics and helps to reduce fermentation time **(Dave and Shah, 1998)**. Donker et al. **(2007)** demonstrated that, supplementation of yoghurt with inulin at low concentration significantly improves the growth and viability of *Lactobacillus acidophilus* and *Lactobacillus casei* used as probiotics in yoghurt during cold storage. Therefore, inulin appears an important food ingredient that would merit to be additionally explored for the production of functional foods.

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J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 6 (8), August, 2015

The yoghurt containing inulin had stable color and water activity and synergensis did not prevail during strong, being similar to the other dietaryfiber containing yoghurt (**Staffolo et al., 2004**). **Pimentel et al. (2013**) reported that, addition of long chain inulin in low fat yogurt could lead to interesting results in sensory properties especially in texture characteristics. In particular, replacement of native milk fats with long chain inulin created equally acceptable firmness and color as with yogurt containing native milk fats. Most of the characteristics of the final product and the process remain fairly close to the values of the originals. Several short chain prebiotics have a slightly negative effect on the firmness and creaminess of the yogurt whereas long chain prebiotics increase those values. Overall, the final choice remains with the consumer and their preference for texture.

Substitutions of wheat flour by non-wheat flour led different in satisfying bread products. However, composite wheat breads generally displayed reduction in , loaf volume and impairment of sensory qualities (e.g. appearance, texture, and flavor), as the level of substitution of wheat with non-wheat flour increased (Peressini and Sensidoni, 2009). Due to the fact that the main selection criteria of wheat are based on their ability to give bulky white breads, it appeared useful to seek products of substitution that had less negative impacts on the volume of the bread. Therefore, it is necessary to have a low content of ashes in the composite flours of cereals or roots used in bread-making process to obtain bread with a pleasant crumb color and taste (Zhang and Moore, 1997). Tiger nut flour could be used in bakery products (Chinma et al., 2010) as well as to formulate gluten-free bread with good baking and nutritional characteristics (Aguilar et al., 2015). Bread elaborated with both chickpea and tiger nut flour maintained its baking characteristics (bake loss, specific volume, crustand crumb color and, crumb hardness) even when shortening and/or emulsifier were reduced or eliminated (Aguilar et al., 2015). Continued increasing interest lies with the polysaccharide inulin. This is due to its nutritional properties and functional abilities. It has been widely utilized as a processing ingredient due to its bulking ability, structure-forming capacity, water retention, and its ability to improve rheological properties and stabilize emulsions and foams (Juszczak et al., 2012).

Clinical trials indicate that viscous soluble fiber is more effective than cereal fiber in lowering serum cholesterol (Kendall et al., 2010). Moreover, dietary intake of viscous fiber has a hypoglycemic effect (Landin et al., 1992). Polaki et al. (2010) and Demirkesen et al. (2013) investigated the effects of using TN flour in a gluten-free formulation in comparison to a rice bread control, the use of tigernut decreased bake loss, increased specific volume, and decreased firmness of the resulting breads when incorporated at the optimal level (10:90/ tigernut: rice). Furthermore, the high level of fat present in TN (20.5%) generated a plasticizing effect on the rheological and proofing properties of the dough, resulting in enhanced crumb structure and bread volume. Inulin is among the most famous prebiotic compounds. In order to improve viability of probiotic bacteria during storage, fermented food should be supplemented with prebiotics. Therefore, this study aims to

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<u>El-Reffaei, W. H. M. et al.</u>

evaluate effect of JR and TN as probiotics 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 (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 slicingsliced cutting (think 0.5 cm) and dried on oven drying at 50 °C until dry, then ground into flour using attrition mill (Globe P44, China) and). The flour samples were passed through a 0.45 mm mesh size sieve _-t 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 until analysis.

Soaking experiments:

Soaking experiments on TN were conducting according to Turhan *et al.* (2002). 400 ml-distilled water put in bBeakers (500 ml), containing 400 ml distilled water which 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-GlobeP44,, 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 JA-JR and TN:

Fat, protein, ash, moisture and fiber contents<u>were</u> 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)

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J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 6 (8), August, 2015

Determination of fatty acids of plants and bio-yoghourts:

Fatty acid profile of the plants and bio-yoghourts, sS amples of each were dried at 60 °C and ground in a coffee grinder to a particle size of approximately 1 mm³ 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 BF₃ 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-waxcolumn. The carrier gas was N2_Helium_with a flame ionization detector, fatty acids identified according to standard FAME.

Determination of polysaccharides:

Determination of polys accharides 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 supernatantwere quantitate transferred to measuring flask 100 ml and completed to the mark HPLC grade water sonicated for one hour, centrifuged for 10 min at 4000 Xg the supernatantwere 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 300x 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 adjustadjusts 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:

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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 HCIO4 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), JA-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 (20032002).

Organoleptic properties:

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J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 6 (8), August, 2015

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 biofedobacterium biofidobacterium and JAJR 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 wheatflour used as control (10072% white wheat flourextraction ratio), JA-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 JA-JR and TN according to AACC (2000) method No (AACC 54-30A). Each Alveograph result was analyzed for the following parameters: Tenacity (P) mm H₂O : 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 (le) %: elasticity index.

Microbiological evaluation: Bacterial strains:

Each Lactobacillus plantarum <u>code no</u>. (ATCC14917) and Lactobacillus curvatus <u>code no</u>. (1136T) strains were obtained from Microbiological Resources Center (Cairo MIRCEN), faculty of Agriculture, Ain Shams University. Staphylococcus aureus <u>code no</u> (A.F 15) and <u>bacillus</u> <u>Bacillus</u> subtilis strains were kindly provided by <u>Dr</u>. Ahmed F. Abdel Salam from , 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, <u>while</u> *Staph aureus* and *Bacillus subtilis* (*B. <u>subtilissub</u>) isolates were maintained through monthly transfer on nutrient agar. All strains were stored at 4 °C.*

Standard inoculums inoculants:

Standard inoculums 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 subsequentenumeration on MRS agar for *L. plantarum* and *L. curvatus*, nutrient agar for *Bacillus subtilis* and Vojel Johnson agar for *Staph aureus*. Plant substances of JA-JR and TN were

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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 10¹³ cfu/ml, then added different concentrations (5.0 and 10.0 %) of JA-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 10¹¹ 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 10¹³ cfu/ml then added different concentrations (5.0 and 10.0 %) of JA-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 <u>JAJR</u>, 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 DISCUSSIONS

Chemical composition of JA-<u>JR</u> and TN:

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The nutrients and proximate analysis of JR and TN are presented in (Table 1). Results revealed that, JR and TN were 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 *etal.* (2014) reported that, the tiger-nut contain from 25.35 to 28.19% fat and 3.28 to 7.32% protein. The predominant constituent in JR <u>carbohydrates was being was</u> 78.00 % and follows by protein as being 9.8%. However, the carbohydrate and follows by <u>crude fat</u>, 41.22 and 35.43, respectively, were the most predominate nutrients constituent in TN.

crude fat, 41.22 and 35.43, respectively, were the most predominate nutrients constituent in TN (Table 1). Soaking process improved chemical composition of TN as presented in table 2.

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

W			JA <u>J</u> R		TN		
	Nutrients	RDV	Contents **	% of RDV	Contents **	% of RDV	
	Protein (g)	50	9.8±0.36	19.6	9.7±0.16	19.40	
1			270				

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J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 6 (8), August, 2015

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r▶ 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. <mark>00</mark> ±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 JA 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, is oleucine, phenylalanine, threonine, valine and lysine with slightly differences between JR and TN. It found 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. However, JR contained the second predominant AA of lysine; leucine follows by valine as 2.9616, and similar ratio of leucine and valine 2.34 g/100g from total essential AA. In Table 32, TN was 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 wealed 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 guality parameters used for protein evaluation (FAOWHO, 1991). The P-

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PER of JA <u>JR</u> and TN was 0.55 and 0.55<u>52</u>, respectively. This study showed that JA <u>JR</u> and TN nutritionally useful quantities of most of the essential amino acids and can serve as food supplements.

Table (2):2. Amino	acids (AA)	content (g/100g protein)	of JA JR and TN
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AA composition	WHO* ideal AA(g/100)	J	R	٦	٢N
	protein)	AA	% WHO	AA	% WHO
	Esse	ential 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-es	sential A A	À		
Aspartic		11.68		13.12	
Glutamic		18.61		17.50	
Serine		3.47		5.83	
▶ Proline		12.96		5.47	
Glycine	1	4.01		6.93	
Alanine		5.11		8.02	
▶ Histidine	1	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 e	fficiency ratio				•

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

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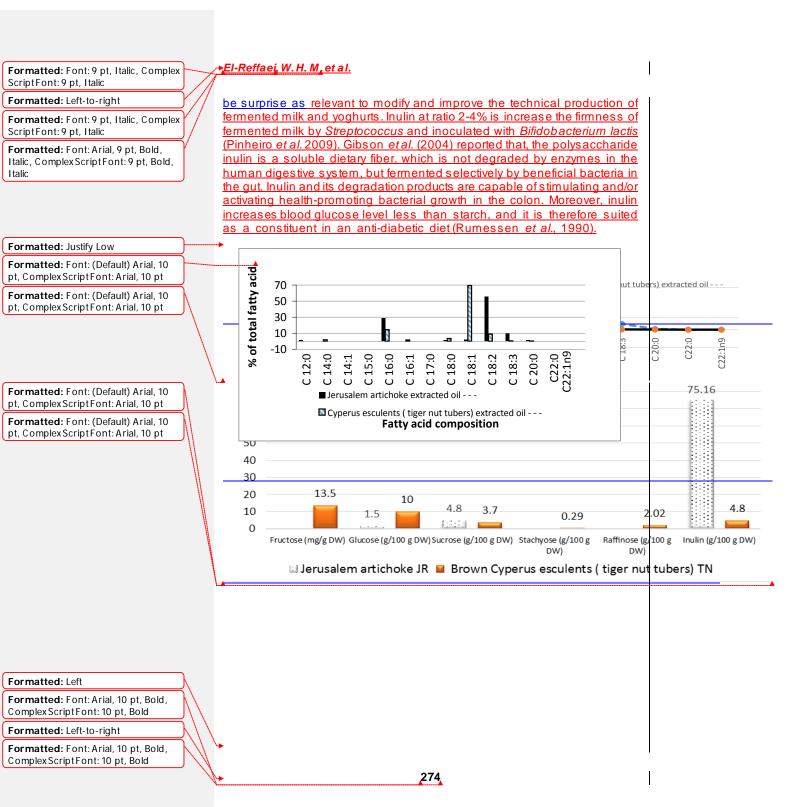
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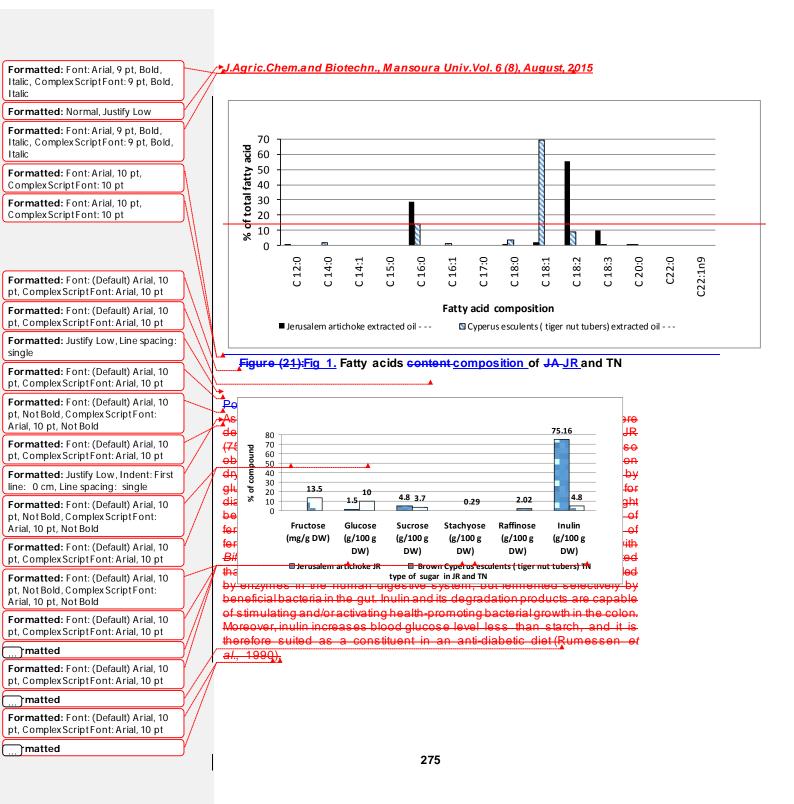
80.11 and 85.67 g/100g protein, respectively. The Predicted Protein Efficiency Ratio (P-PER) is one of the quality parameters used for protein evaluation (FAO/WHO, 1991). The P-PER of JR and TN was 0.55 and 0.52, respectively. This studyshowed that JR and TN nutritionally useful quantities of most of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

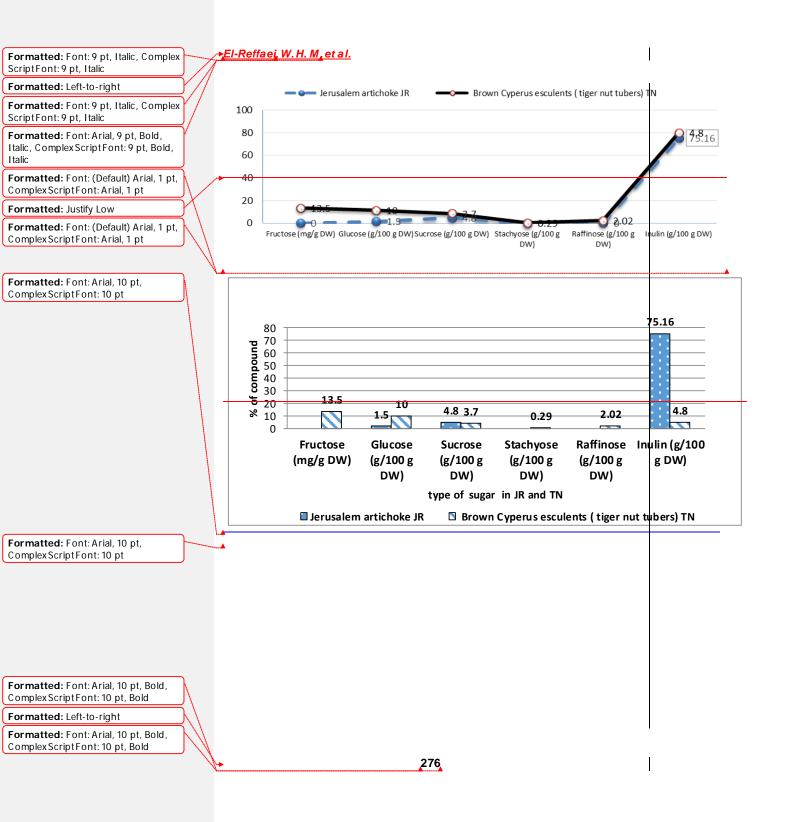
Figure (1 Fig 1.) illustrated fatty acids composition content of crude lipid fraction from JA-JR and TN. The results revealed that, JR fatty acid profile is predominant in linoleic acid, c18:2n6 (55.34 %) and follows by palmitic acid, C16:0 (29.04%) and linolenic acid, C18:3n3 (9.6%), these account more 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







J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 6 (8), August, 2015

Figure (2):Fig 2. Polysaccharides content in Jerusalem artichoke and tiger nut tubers

Vitamins content in JA_JR and TN:

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The mostabundant vitamin compound in JAJR 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 etal. (2007).

with those of 14 except for Vit B2 both plants seem capable of satisfying less than 6% of an arbit's requirement for the various nutrients listed in Table (a). The TN provided an adaptive quantity of Vitamire E and B1 about 51 and 21%, respectively varius than PDV recommandation. A quite similar vitamin context in 14 when compared to TN vitamin E and Vit B1 accounted 47.33 and 13.27 as % of RDV, respectively. The obtained results were

Table (3):3. Vitamins conte	nt in JA and TN (100	g dry base)	
	JA <u>JR</u>	TN	

2	Vitamins	RDV <u>+</u>	Contents **	% of RDV	Contents **	% of
≻				· · · · ·		RDV
1	Vit B1 (mg)	1.50	0.199 ±0.48	13.27	0.31±0.35	20.67
1	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
11.						

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

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).

Minerals content in JA_JR and TN:

Minerals content in JA-JR and TN illustrated in (Table 4), JA-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 JA-JR provide a sufficient amount more than quarter ratio of copper USDA recommend. Both of JA-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.*,

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) matted) matted) matted) matted) matted) matted 2009). Iron is necessary for the prevention of anemia, and zinc necessary for nucleic acid metabolism protein synthesis and cell growth (lgoe, 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):4. Mineral contents of JA-JR and TN.

1				Min	nerals <u>n</u>	ng/kg				
1		Ca	Р	Mg	Мо	Fe	Zn	Cu	Mn	S
*	JA JR	120	370	1127	11.92	117.2	17.17	5.35	10.84	1250
•	TN mg/kg	150	330	1323	9.75	131.5	12.11	ND	11.39	1369
F	RDV-+	1000	1000	400	75	18	15	2.0	2.0	_
1	<u>(mg)</u>	mg	mg	mg	mg	mg	mg	mg	mg	_

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

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).

GC-MS analysis in JA-JR and TN:

Data from the analysis of JA-JR and TN volatile components are illustrated in Table (5). It showed that, 20 compounds in JAJR, 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 JA-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 I-(+)-As corbic 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 etal. (2005) who reported that, TN contained high amounts were p-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

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J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 6 (8), August, 2015

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 y-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 *etal.*, 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 *etal.* 2001).

Fatty acid (FA) composition of bio-yoghourt:

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Table (6):6. Fatty acid composition of bio-yoghourt fortified with JA-<u>JR</u> or TN flour.

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

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EI-Reffaei, W. H. M, et al.

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

The instrumental texture profile of bio-yoghurt fortified with 10% of JAJR 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 8.21 and 9.22 for JA-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 JA-JR and TN bio-yoghurt. The stability of texture profile is-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.

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

	Texture profile	Control	Fortified with JA <u>JR</u>	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):8. Organoleptic evaluation for bio yogurts fortified with 10% JR or TN

1	<u>Taste parameter</u>	<u>Control</u>	<u>Fortified with</u> <u>biofedobacterium</u> <u>and JR</u>	Fortified with biofedobacterium and TN
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J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 6 (8), August, 2015

×	<u>Sourness</u>	<u>6.3±1.9</u>	<u>6.5±2.2</u>	<u>5.3±1.2</u>
1	<u>Bitterness</u>	<u>4.2±1.2</u>	<u>3.1±0.7</u>	<u>2.2±1.6</u>
1/e	<u>Sweetness</u>	<u>2.6±1.6</u>	<u>4.3±1.5</u>	<u>5.7±1.0</u>
ŀ	<u>Aroma</u>	6.3 ± 0.8	<u>4.2±1.7</u>	<u>5.8±2.1</u>
	<u>Overall</u> acceptability	<u>6.7±0.2</u>	<u>5.3±1.8</u>	<u>7.1±1.7</u>

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.

Organoleptic evaluation for bio- yogurts:

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Results from Table (8) showed organoleptic evaluation of bio-yoghurt fortified with 10% of JA-JR or TN. Sourness was decrease in fortified TN. The TN bio-yoghourt was lower in brightness than control and JA-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 JA-JR yoghurts. Bio-yoghurt. There was a defiantly higher overall acceptability in TN than control and JA-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 sweetflavor.

 Table (8): Organoleptic evaluation for bio yogurts fortified with 10% JA or TN

1	Taste		Contro	Fortified with	For tified with
parameter		4		biofedobacterium and JA	biofedobacterium and TN
/>	Sourness	1.9	6.3±	6.5±2.2	5.3±1.2
•	Bitterness	1.2	4 <u>.2±</u>	3.1±0.7	2.2±1.6
•	Sweetness	1.6	2.6±	4 .3± 1.5	5.7±1.0
•	Aroma		6.3±	4 .2± 1.7	5.8± 2.1
+ ac	Overall ceptability	0 <u>.2</u>	6.7±	5.3±1.8	7.1±1.7

Rheological properties of dough:

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The results obtained from alveographic measurement of wheat flour (WF) substituted with JA and TN summarized in Table (9). Substitute WF with 5, 10 and 15% of JA 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, which is essential for the production of bakery products with a light texture. Whereas, doughs prepared from these flours at different ratio of substitutions did not have optimal viscoelastic <u>Table (9): Rheological properties of</u> <u>wheat flour (WF) dough supplemented with different concentration of</u> JR or TN

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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 JA_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 JA_JR_or TN has produced worst quality of dough elasticity and extensibility. This result is worth mentioning that addition ratio of JA_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.*

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J.Agric.Chem.and Biotechn.	, Mansoura	Univ.Vol. 6	(8), August, 2015

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SL	<i>ıbtilis</i> in e	enrichmen	tbroth	medium	and	following	byincr	easing	counts	of L.
pl	antarum	from 4×1	<u>0¹³ to</u>	<u>3× 10^{1/} ,</u>	<u>L.</u>	-				

+ <u>Flour</u> alveograph	<u>WF</u>		<u>JA</u>			<u>TN</u>	
properties	<u>100%</u>	<u>5%</u>	<u>10%</u>	<u>15%</u>	<u>5%</u>	<u>10%</u>	<u>15%</u>
<u>Tenacity (P)</u> mm H ₂ O	<u>85.7</u>	<u>76.0</u>	<u>54.0</u>	<u>38.00</u>	<u>89.0</u>	<u>44.00</u>	<u>46.00</u>
Extensibility (L) mm	<u>53.1</u>	<u>46.0</u>	<u>41.0</u>	<u>29.00</u>	<u>29.0</u>	<u>32.0</u>	<u>18.0</u>
<u>Index of</u> swelling (G)	<u>17.2</u>	<u>15.1</u>	<u>14.3</u>	<u>12.0</u>	<u>12.0</u>	<u>12.6</u>	<u>9.4</u>
<u>Baking</u> <u>strength</u> (W)10E-4 J	<u>160.8</u>	<u>151.0</u>	<u>94.00</u>	<u>46.0</u>	<u>113.0</u>	<u>57.00</u>	<u>36.0</u>

<u>curvatus</u> from 4×10^{13} to 4×10^{17} and <u>B</u>. subtilis from 4×10^{11} to 4×10^{16} cfu/ml, while TN at concentration 10% increased counts of <u>L</u>. plantarum from 4×10^{13} to 8×10^{16} <u>L</u>. curvatus from 4×10^{13} to 4×10^{17} and <u>B</u>. subtilis from 4×10^{11} to 13×10^{16} <u>cfu/ml</u> 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
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 (WF)
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 supplemented with different concentration of JR or TN

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$\leq 1/2$	Configuration rate (P/L) %	<u>1.77</u>	<u>1.65</u>	<u>1.32</u>	<u>1.31</u>	<u>3.07</u>	<u>1.38</u>	<u>2.56</u>
\mathbb{R}	<u>Elasticity</u> index (le) %	<u>75.6</u>	<u>60.00</u>	<u>51.4</u>	<u>45.20</u>	<u>55.6</u>	<u>43.8</u>	<u>36.15</u>
	Table 10. Eff	ect of J	R and TN	on som	e probiot	tic bacte	ria count	ts in vitro

Treatments*

	1100	unento.						
with out	J	<u>R</u>	TN					
▶ <u>without</u>	<u>5%</u>	<u>10%</u>	<u>5%</u>	<u>10%</u>				
► <u>L. plantarum</u> 7 ×10 ¹³ cfu/ml	<u>2 × 10¹⁵</u>	<u>3 × 10¹⁷</u>	<u>3 × 10¹⁶</u>	<u>8 × 10¹⁶</u>				
► <u>L. curvatus</u>	<u>3 × 10¹⁶</u>	<u>4 × 10¹⁷</u>	<u>5 × 10¹⁶</u>	<u>4 × 10¹⁷</u>				
$\blacktriangleright \underline{B. subtilis}_{0 \times 10^{11} \text{ cfu}/\text{m}}$	<u>5 × 10¹⁴</u>	<u>4 × 10¹⁶</u>	<u>10 × 10¹⁴</u>	<u>13 × 10¹⁶</u>				
<u>0 x 10 010/111</u>								
*The used inoculum of <i>L. plantarum</i> was 4×10 ¹³ cfu/ml; <i>L. curvatus</i> was 4×10 ¹³ cfu/ml								
and <i>B. subtilis</i> wa	as 4× 10 ¹¹ cfu/ml							

Microbiological evaluation:

Effect of JAR and TN on some probiotic bacteria in vitro:

The results recorded in Table (10) clearly showed that JAR at concentration 10% encouraged growth of *L. plantarum* P ; *L. curvatus* L.C and *B. subtilis* sub in enrichment broth medium and following by increasing counts of *L. plantarum* LP from 4×10^{12} to 3×10^{12} , *L.* LC from 4×10^{12} to 4×10^{12} and BS from 4×10^{12} to 4×10^{12} cfu/ml, while TN at concentration 10% increased counts of *L.P.* from 4×10^{12} to 8×10^{12} , *L.C.* from 4×10^{12} to 4×10^{12} and *B. sub* from 4×10^{12} to 13×10^{12} to 8×10^{12} , *L.C.* from 4×10^{12} to 4×10^{12} and *B. sub* from 4×10^{12} to 13×10^{12} gfu/ml in enrichment broth medium. The concentration 10% of JA 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 JA or TN, improve carbohydrate content and prebiotic inulin, which is contribute to increase activity of *Lactobacillus plantarum* and *Lactobacillus curvatus* than control yoghurt. This also recorded by **Buriti et al. (2010a)** and **Komatsu et al., 2013**.

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

Microbiological evaluation:

Effect of JA and TN on some probiotic bacteria in vitro:

The results recorded in Table (10) clearly showed that JA at concentration 10% encouraged growth of L.P; L.C and B. sub in enrichment broth medium and following by increasing counts of LP from 4×10^{13} to 3×10^{17} , LC from 4×10^{19} to 4×10^{17} and BS from 4×10^{14} to 4×10^{17} cfu/ml, while TN at concentration 10 % increased counts of *L.P.* from 4×10^{19} to 8×10^{10} *L.C.* from 4×10^{19} to 4×10^{17} and *B. sub* from 4×10^{14} to 13×10^{19} cfu/ml, in enrichment broth medium. The concentration 10 % of JA or TN

J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 6 (8), August, 2015

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Table (10): Effect of JA JR and TN on some probiotic bacteria counts in vitro

1	1110				
		Treatments	<u>*</u>		
	-	JA <u>JR</u>		- TN	
	Control <u>without</u>	5%	10%	5%	10%
	► <u>L. plantarum</u> L.P 7 ×10 ⁴⁹ cfu/ml	2 × 10 ¹⁵	3 × 10¹⁷	3 × 10 ¹⁶	8 × 10 ¹⁶
7	<u>L. curvatus</u> L.C	3 × 10 ⁴⁶	4 × 10 ⁴⁷	5 × 10¹⁶	4 × 10 ¹⁷
1	<u>→B. subtilis</u> B. sub	5 × 10 ¹⁴	4 × 10 ¹⁶	10 × 10¹⁴	

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

*The used inoculum of <u>L. plantarum</u>L. P was 4× 10⁴³cfu/ml; <u>L. curvatus</u>L. C was 4× 10⁴³cfu/ml and <u>B. subtilis</u>B. sub was 4× 10⁴⁴cfu/ml

<u>curvatus from 4x 10¹³ to 4x 10¹² and B. subtilis from 4x 10¹¹ to 4x 10¹⁶ cfu/ml, while TN at concentration 10 % increased counts of L. plantarum from $4x 10^{12}$ to $8x 10^{16}$ L. curvatus from $4x 10^{12}$ to $4x 10^{12}$ and B. subtilis from $4x 10^{12}$ to $8x 10^{16}$ L. curvatus from $4x 10^{12}$ to $4x 10^{12}$ and B. subtilis from $4x 10^{12}$ to 10^{12} to $13x 10^{10}$ 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</u>

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

 Effect of JA-JR and TN on growth and survival of Staph aureus in vitro: The obtained results in Table (11) showed that, JA-JR and TN at concentration 10.0 % resulted in decrease of Staph aureus counts from 7× 10¹³ to 6× 10⁷ and 5× 10⁶ cfu/ml, respectively in vitro and to 9× 10⁴ and 7× 10° cfu/g, respectively in yoghurt. On the other hand, non-fortified yoghurt samples showed reduced Staph aureus counts from 7× 10¹³ to 2× 10⁹ cfu/g, this these results in agreement with the studies of Mattila –Sandholm et al. (2002) and Dimitroglou et al. (2011) which recorded that, probiotics are 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

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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 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 oligofructos e at the end of storage (Lankaputhra *et al.* 1996).

agents or vaccines. 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). The supplementation of Jerusalem artichoke inulin resulted in greater growth rates of *Lactobacillus casei* than *Bifidobactorium 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).

Table (11): Effect of <u>JA_JR</u> and TN on growth and survival of *Staph* aureus *in vitro

		reatments*			
Control without	J 	<u>JR</u>	TN		
Control <u>w Ithout</u>	5.0%	10.0%	5.0%	10.0%	
<i>In vitr</i> o 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	
		13	1	1	

The used inoculum for Staph aureus was 7 x 10¹³ cfu/ml

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

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<u>bifidobacteria</u> and lactobacilli (Akalin <u>et al.</u> 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 casoi* growth during formentation 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).

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.

<u>Moreover, probiotics can inhibit pathogen multiplication, where low</u> numbers of Lactobacillus delbrueckii would help the survival of probiotic organisms due to reduced risks of post acidification by Lactobacillus delbrueckii

<u>(Shah, 1995). In addition, inulin powder improved Lactobacillus casei growth</u> <u>during fermentation and their survival during storage time (Aryana and</u> <u>McGrew, 2007). The supplementation of Jerusalem artichoke inulin resulted</u> <u>in greater growth rates of Lactobacillus casei than Bifidobacterium bifidum</u> <u>and Lactobacillus acidophilus during cold storage of yoghurt (Paseephol and</u> <u>Sherkat, 2009). The mechanism by which inulin improve the viability of the</u> <u>probiotic organisms during cold storage is still unclear, while the two possible</u> <u>mechanisms proposed so far state that inulin's provide additional nutrients for</u> <u>promoting culture growth (Makras *et al.*, 2005), and that they protect probiotic <u>cells from acid injury (Desai *et al.*, 2004).</u></u>

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 JA-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 JA-JR and TN **291**

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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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matted	\mathbf{k}	المصادر الشكرية تكر من لبنى الطرطونة وحب الغرير للمصادر لأولية للريبيوليك وتعيم مادم إلتتاجها ونموها في الأاغذية المتخمرة كالزبادي والعجائن و مدي تاأثير ها على حفظ تلك المنتجات من
		خلال تقييم جويدتها المختلفة وكذلك مستوي البكتريا الممرضية في تلك الاأغذية ومدي إليقاف وتثبيط
		بكتريا الإلاستاف الممرضة. ويعد الألانيولين المتوافر في الطرطوفة من لأهم المواد التي لها دور

El-Reffaei, W. H. M, et al.

وظيفي كبريبيونيك مسببةلنشاط البكتريا المفيدة صحيا وتغذويا.وكنلك تم التطرق اللي كل مكونا<u>ت.</u> تلك النباتات كيماويا وتحديد المغذيات الكبري والصغري من مطحوتها بعد معاملة <mark>حب العزيز بالنقع.</mark> والتجفيف.<u>-</u>

تم تقدير محتوا هما من اللألحماض الأمينية والأحماض الدهنية والتحليل الكروماتوجرافى الكتلى وتولركيب السكريات المختلفة واستخدم الضافات للعجان بنسب مختلفة من كلا الدرنات بعد طحنها فى النتاج عجان تتميز بانخفاض مستوى الجلوتين الذي يناسب بعض المرضي سواء مرضى للسكر الو مرضضي السيلك . وقد تم التحاليل الميكروبيولوجية على البكتريا سواء المنشطة من البروبيوتيك او الممرضة والمسية لفساد الأغذية من بكتريا الإستاف للاستلف فى الزبادي المدعم بكلا المصادر من الدرنات محل الدراسة . وقد اظهرت الدراسة إلى من المكنية الستخدام تلك الدرنات فى البحاد الأغذية وظيفية لبعض المرضى والحالات الغذائية فيضلا عن اليحاد مصدر القتصادي لتك ألاغذية الغيبة.

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