Poster Session | Food Function/Nutrition

# [6-1130-P] Functional/Wellness Foods &Nutrition (6th)

Fri. Sep 6, 2019 11:30 AM - 12:30 PM Poster Place (Entrance Hall)

## [6-1130-P-01] Primary Prebiotic Properties of Ethanolic Sugar Extract from Groundnut Seeds

*Pairote Wongputtisin[1], Narin Lahsom[1] (1. Program in Biotechnology, Faculty of Science, Maejo university, Chiang mai, Thailand (Thailand))

Keywords: Groundnut, Arachis hypogaea, Raffinose Family Oligosaccharides, Prebiotic, Probiotic, Functional Food

Raffinose family oligosaccharides (RFOs) have been accepted as an effective prebiotic substance. They can be generally found in various leguminous seeds. Thus, legume seeds can be considered as promising sources of prebiotic ingredient for development of functional foods. The aims of this work were analysis of RFOs composition in local groundnut (*Arachis hypogaea* L.) of Thailand and primary investigation for their prebiotic potential. In this study, low molecular weight sugars (LMWSs) including RFOs were extracted from seeds of three local groundnut cultivars in Thailand, i.e. Tainan 9, Khonkean 5 and Khonkean 6, using 50% (v/v) ethanol. LMWSs were qualified and quantified by HPLC apparatus and subsequently investigated for their capacity in growth stimulation of some enteric bacteria. The results showed that these cultivars contained LMWSs approximately 28-40 mg/g dry seed and the average size of sugars in term of degree of polymerization (DP) ranged between 2 and 7. These seeds contained low amount of raffinose and verbascose, while high amount of stachyose was found at 3.9-11.7 mg/g dry seed. Growth of probiotic *Lactobacillus acidophilus* TISTR1338, *L. plantarum* TISTR541 and *L. lactis* TISTR1464 were stimulated significantly in basal media containing groundnut LMWSs ($p<0.05$), while growth of *Salmonella enterica* serovar Typhimurium TISTR292 and *Escherichia coli* were not stimulated. Interestingly, growth of *S.* Typhimurium and *E. coli* were suppressed when was co-cultured with those *Lactobacillus* sp. in basal media contained groundnut LMWSs as a carbon source. Thus, it might be concluded that ethanolic sugar extracted from seeds of Tainan 9, Khonkean 5 and Khonkean 6 exhibited the primary properties to be accepted as prebiotic substance.

# Primary Prebiotic Properties of Ethanolic Sugar Extract from Groundnut Seeds

Pairote Wongputtisin* and Narin Lahsom
Program in Biotechnology, Faculty of Science, Maejo University, Chiang mai, Thailand 50290

*corresponding author: pairotewong@gmail.com

## ABSTRACT

Raffinose family oligosaccharides (RFOs) have been accepted as an effective prebiotic substance. They can be generally found in various leguminous seeds. Thus, legume seeds can be considered as promising sources of prebiotic ingredient for development of functional foods. The aims of this work were analysis of RFOs composition in local groundnut (*Arachis hypogaea* L.) of Thailand and primary investigation for their prebiotic potential. In this study, low molecular weight sugars (LMWSs) including RFOs were extracted from seeds of three local groundnut cultivars in Thailand, i.e. Tainan 9, Khonkean 5 and Khonkean 6, using 50% (v/v) ethanol. LMWSs were qualified and quantified by HPLC apparatus and subsequently investigated for their capacity in growth stimulation of some enteric bacteria. The results showed that these cultivars contained LMWSs approximately 28-40 mg/g dry seed and the average size of sugars in term of degree of polymerization (DP) ranged between 2 and 7. These seeds contained low amount of raffinose and verbascose, while high amount of stachyose was found at 3.9-11.7 mg/g dry seed. Growth of probiotic *Lactobacillus acidophilus* TISTR1338, *L. plantarum* TISTR541 and *L. lactis* TISTR1464 were stimulated significantly in basal media containing groundnut LMWSs ($p<0.05$), while growth of *Salmonella enterica* serovar Typhimurium TISTR292 and *Escherichia coli* were not stimulated. Interestingly, growth of *S.* Typhimurium and *E. coli* were suppressed when was co-cultured with those *Lactobacillus* sp. in basal media contained groundnut LMWSs as a carbon source. Thus, it might be concluded that ethanolic sugar extracted from seeds of Tainan 9, Khonkean 5 and Khonkean 6 exhibited the primary properties to be accepted as prebiotic substance.

**Keywords:** Groundnut, *Arachis hypogaea*, Raffinose Family Oligosaccharides, Prebiotic, Probiotic, Functional Food

## 1. INTRODUCTION

Raffinose family oligosaccharides (RFOs) are oligosaccharides widely found in leguminous seeds. They are α-galactosyl derivative of sucrose linked with α(1→6) bond. The major member of RFOs are raffinose, stachyose and verbascose, which their chemical structures are shown in Figure 1. Biosynthesis of raffinose in legume seeds proceeds by transferring of galactosyl residue (donor) from galactinol (*O*-α-D-galactopyranosyl-(1→1)-L-*myo*-inositol) to sucrose (acceptor) by the action of raffinose synthase. Subsequently, stachyose synthase transfers another one or two galactosyl residue from galactinol to raffinose molecule, resulting of stachyose and verbascose, respectively (Peterbauer et al., 2002; Karner et al., 2004). The RFOs content in various leguminous seeds; i.e. soybean, lupin, chickpea, mung bean, pigeon pea, jack bean, lentil and groundnut has been reported (Muzquiz et al., 1999; Kadlec, 2001; Martinez-Villaluenga et al., 2005; Giannoccaro et al., 2006; Xiaoli et al., 2008; Kumar et al., 2010). In case of groundnut (*Arachis hypogaea* L.), variation of RFOs in different cultivars was reported by other research groups. However, those of local groundnut cultivars in Thailand have not been investigated yet.

These sugars play an important role in seed by involving in defense mechanism of some abiotic stresses; low temperature, drought, high salinity and oxidative stress (ElSayed et al., 2014). However, these oligosaccharides have been reported as an effective prebiotic substance for human and animal too. The term "prebiotics" was firstly introduced by Gibson and Roberfroid in 1995 and presently, its definition has been modified, for example "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health (Gibson et al., 2004)" and "live micro-organisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2002)". According to these concepts, non-digestible oligosaccharides (NDOs) such as fructooligosaccharide (FOS), galactooligosaccharide (GOS), isomaltooligosaccharide (IMO), xylooligosaccharide (XOS), human milk oligosaccharide (HMO) and raffinose family of oligosaccharides (RFO) are accepted as prebiotic (Ziemer and Gibson, 1998; Chow, 2002; Mussato and Mancilha, 2007).

There were some evident that groundnut originated from South America before spreads to other regions, including Thailand. Groundnuts, cultivar Tainan9, Khonkean5 and Khonkean6 are the examples of popular and widespread groundnuts in Thailand. In this study, RFOs composition in seed of these cultivars were quantified. Subsequently, primary prebiotic properties of seed extract containing RFOs were investigated, with respect to growth stimulation ability to 3 probiotics strains; i.e. *Lactobacillus lactis, L. acidophilus and L. plantarum,* and also normal flora *Escherichia coli* and pathogenic *Salmonella* Typhimurium. The aim of this study was to introduce the prebiotic property of local groundnuts from Thailand, the other functionality apart from consuming as a protein and oil food.

## 2. METHODOLOGIES

### 2.1 Groundnut seeds

Seeds of three groundnut cultivars; Tainan 9, Khonkean 5 and Khonkean 6, were kindly obtained from Field Crop Research Center, Thailand, and stored in vacuumed plastic bag at 4 ºC.

### 2.2Microorganisms

All tested bacteria were from the Thailand Institute of Scientific and Technological Research (TISTR). There are totally three probiotic strains; including *Lactobacillus plantarum* TISTR541, *L. lactis* TISTR464 and *L. acidophilus* TISTR1338. The normal flora and pathogenic strains used in this study were *Escherichia coli* TISTR887 and *Salmonella enterica* serovar Typhimurium TISTR292, respectively. Probiotics were maintained on MRS agar, while *E. coli* and *S.* Typhimurium were maintained on nutrient agar.

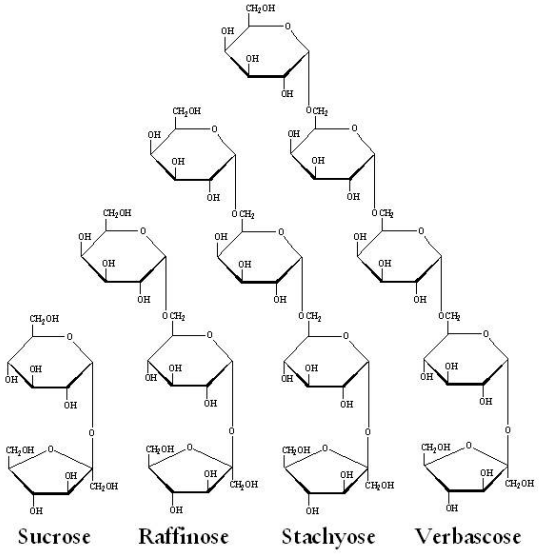


Figure 1. The chemical structures of raffinose family of oligosaccharides (RFOs)

**2.3 RFOs-rich extract preparation**

The crude extract containing low molecular weight sugar (LMWS) and rich of RFOs was prepared from ground and dried seeds according to the modified method of Xiaoli et al. (2008). Ground seed was defatted using hexane and mixed with 50% (v/v) ethanol with the ratio of 3 g : 50 ml. The mixture was continuously shaken for 1 hr at 30 $^{o}$C and then filtered through filter paper (Whatmann® No. 1). The obtained filtrate was subsequently centrifuged at 8,000 rpm for 10 min at 4$^{o}$C to remove the remaining particles. Supernatant was concentrated using rotary vacuum evaporator (Buchi®) under the temperature below 50$^{O}$C.

**2.4 Analysis of sugars**

Reducing sugar and total sugar and in the extract was determined by DNS and phenol-sulfuric acid method; respectively. Size of sugar, in term of an average degree of polymerization (DP) was calculated by the ratio between total sugar and reducing sugar content. Quantity of some LMWSs were analyzed using high performance liquid chromatography (HPLC) apparatus, consisting of 5 μm Previal Amino column (Alltech®), series III HPLC pump and Evaporative Light Scattering Detector (ELSD) (Alltech®). The column temperature was controlled at 30±1 $^{o}$C during analysis. Acetonitrile: deionized water (75: 25) was used as mobile phase at the flow rate of 1.0 ml/ min. The injection volume was 20 μl and all samples were filtered through nylon membrane (VERTICAL®) (0.45 μm) prior injection. HPLC grade of glucose (Fluka®), sucrose (Fluka®), raffinose (MERCK®), stachyose (ALDRICH®) and verbascose (Fluka®) were used as standard sugars.

**2.5 Primary prebiotic properties of RFOs-rich extracts**

Growth stimulation of individual bacteria by groundnut sugar extracts were investigated. The extract was supplemented in basal medium (g/ L: 0.3 $K_2HPO_4$, 0.1 $KH_2PO_4$, 1.0 yeast extract, 1.0 peptone, 0.2 $MgSO_4$, and 2.5 $(NH_4)_2SO_4$, pH 7.0) as a carbon source at a concentration of 1% (w/v). Approximately $10^8$ CFU of 24 hr-old inoculum of tested bacterium was transferred to 100 ml sterilized basal medium and statically incubated in anaerobic jar for 24 hr and at 37$^{o}$C. Viable cell (CFU/ml) of probiotics, *Sal.* Typhimurium and *E. coli* was enumerated on De Man, Rogosa and Sharpe agar (MRS) (Himedia®), *Salmonella – Shigella* agar (SS agar) (Himedia®) and Eosin methylene blue agar (EMB agar) (Himedia®); respectively.

The growth dynamic of each bacterium in defined-mixed culture was also studied. Total $10^8$ CFU of 3 probiotic strains (~3.3 x $10^7$ CFU for each strain), $10^8$ CFU of *S.* Typhimurium and $10^8$ CFU of *E. coli* were transferred as a mixed inoculum to 100 ml basal medium supplemented with groundnut sugar extract. The culture conditions were as described in previous experiment. The bacterial population were monitored

at 0, 12 and 24 hr of cultivation. In both experiment, basal media with glucose as a carbon source and without carbon source were used as control treatments.

**2.6 Statistical analysis**

All experiments were performed in triplicate. STATISTIX© software version 9 was used to analyze the significant difference between treatments.

## 3. RESULTS AND DISCUSSION

### 3.1 Sugar analysis

The ethanolic sugar extract from seed of three groundnut cultivars composed different amount of soluble LMWSs between 2.82-4.00 g/ 100g dry seed, while soluble reducing sugar contents were between 0.48-1.71 g/ 100g dry seed. Then, the average size of LMWSs from all cultivars in term of DP were found in the range of short chain oligosaccharides (Table 1). The results from HPLC were also showed that these three groundnut seeds contained low amount of raffinose and verbascose, while high amount of stachyose was found at 0.39-1.17 g/ 100g dry seed. Moreover, low molecular weight; i.e. glucose and sucrose were also detected (Table 1). Sucrose was found in these groundnuts with remarkably large proportion similar to other groundnut cultivars previously reported as shown in Table 2, correlating to their sweet attributes. Comparing to other leguminous seeds, sucrose and total RFOs composition were not much different (Muzquiz *et al*., 1999; Ekvall et al., 2007; Xiaoli *et al*., 2008; Saldivar et al., 2010; Wongputtisin et al., 2015). However, content of these sugars can be variable, depending on genetic and environmental factors, i.e. vegetation time, storage time, temperature and packaging as earlier reported in lupin and soybean seed by Trugo et al. (1988) and Saldivar et al. (2010).

### 3.2 Prebiotic properties of ethanolic extract from groundnut seeds

The results showed that growth of three probiotic strains were promoted after 24 hr cultivation in broth supplemented with ethanolic extract containing RFOs from groundnut seeds ($p<0.05$), especially *L. lactis*, as shown in Figure 2. Considering on the basal medium with glucose, the most common monosaccharide for microorganism to utilize, we found lesser growth than using groundnut extract as carbon source. On the other hand, groundnut extracts did not promote growth of *S.* Typhimurium and *E. coli* (Figure 2). We also found the obvious inhibitory effect on growth of *E. coli* by the extracts of Khonkean5 and Khonkean6 ($p<0.05$).

Table 1. Sugar content in seeds of three cultivars of groundnut, Tainan 9, Khonkean 5 and Khonkean 6

| Soluble sugar content | Cultivars | | |
|---|---|---|---|
| (g/ 100 g dry seed) | Tainan 9 | Khonkean 5 | Khonkean 6 |
| total sugar | 3.63±0.46 | 4.00±0.30 | 2.82±0.47 |
| reducing sugar | 1.71±0.24 | 0.56±0.15 | 0.48±0.13 |
| degree of polymerization | 2.2 | 7.0 | 5.8 |
| glucose | 0.16±0.04 | 0.09±0.07 | trace |
| sucrose | 1.48±0.05 | 1.32±0.33 | 0.88±0.17 |
| raffinose | 0.01±0.02 | 0.04±0.04 | trace |
| stachyose | 0.56±0.034 | 1.17±0.17 | 0.39±0.079 |
| verbascose | trace | 0.05±0.05 | trace |

Table 2. comparison of LMWSs detected in some leguminous seeds

| Legumes | Content (g/ 100g seed) | | | | |
|---|---|---|---|---|---|
| | Sucrose | Raffinose | Stachyose | Verbascose | References |
| *Groundnut* | | | | | |
| 60 Spanish cultivars | 2.44 – 7.61 | 0.17 – 1.56 (total RFOs) | | | Bishi et al. (2013) |
| 40 Indian cultivars | 2.61 - 6.50 | 0.01 - 0.12 | 0.11 - 0.67 | 0.00 - 0.07 | Bishi et al. (2014) |
| 30 Spanish cultivars | 2.79 – 5.33 | 0.02 – 0.06 | 0.35 – 0.79 | No report | Mahatma et al. (2016) |
| 30 Virginia cultivars | 3.85 – 6.90 | 0.04 – 0.16 | 0.46 – 1.03 | No report | Mahatma et al. (2016) |
| 3 Thai cultivars | 0.81 – 1.48 | 0.00 – 0.04 | 0.39 – 1.17 | 0.00 – 0.05 | This study |
| *Soybean* | | | | | |
| Chiang mai60 | 1.32 | 0.67 | 14.53 | 0.16 | Wongputtisin et al.(2015) |
| V95-7456 | 4.96 | 0.64 | 3.77 | No report | Saldivar et al. (2010) |
| *Vine pea* | No report | 0.29 | 0.14 | 0.13 | Ekvall et al. (2007) |
| *Lupin* | 16.2 | 19.0 | 54.0 | 10.8 | Muzquiz *et al*. (1999) |
| *Chick pea* | 2.56 | 0.89 | 2.38 | 0.42 | Xiaoli *et al*. (2008) |

The change in population of total probiotics, *S*. Typhimurium and *E. coli* in defined-mixed culture experiment were illustrated in Figure 3. It was found that survivability of all bacteria declined along with cultivation time in broth without carbon source, while growth of total probiotic and *E. coli* increased non-significantly and that of *S*. Typhimurium was not significantly changed when glucose was used as carbon source. The interesting results were found in treatment of Tainan9 extract addition. Sugar extract of this cultivar was able to promote growth of total probiotics, resulting in decreasing of *E. coli* and *S*. Typhimurium survivals markedly. Sugar extracts of Khonkean5 and Khonkean6 also gradually enhanced total probiotic growth but not obviously different. However, inhibitory effect on growth of *E. coli* and *S*. Typhimurium still could be observed. The growth pattern of probiotic strains and *S*. Typhimurium in media with groundnut sugar extracts were consistent with the results from single culture study.

From all results above, it was clear that sugar extract from groundnuts could stimulate all tested probiotic strains but not for *E. coli* and pathogenic *S.* Typhimurium. This characteristic is considered as an important primary property prior accepted as prebiotic substance. Probiotic growths could be from both RFOs, which were major sugars in the extract, and the other LMWSs; i.e. glucose and sucrose. To utilize RFOs, bacterial cell required α-galactosidase to hydrolyze α linkage and raffinose delivery system into cell. Mechanisms of RFO utilization in Bifidobacterium and Lactobacilli probiotics were also reported by Hachem et al. (2012). Glycoside hydrolase family 36 (GH36) α-galatosidase encoding genes, sugar transport systems of the glycoside – pentoside – hexuronide cation symporter family (GPH), sugar phosphotransferase systems (PTSs) or ATP-binding cassette systems (ABCs) are key factors. Schmid and Schmitt (1976) reported that *E. coli* cells lack of raffinose delivering system. Moreover, there have been no report on the activity of α-galactosidase in *S*. Typhimurium and *E. coli*, while that was reported in three Lactobacilli used in this study (Donkor et al., 2007; Sumarna, 2008; LeBlanc et al., 2004; Fredslund et al., 2011; Silvestroni et al., 2002; Jeong et al., 2008). Thus, there was high possibility that growth of *E. coli* and *S.* Typhimurium observed in this work were from LMWSs not from RFOs. The expected results were obtained in media added by groundnut sugar extracts. Promoted probiotic population subsequently exhibited the inhibitory effect on *E. coli* and *S*. Typhimurium growth. The mechanisms involved might be commonly explained that lactic acid bacteria produce various inhibitors, for example, organic acids (lactate and acetate), short chain fatty acids, hydrogen peroxide and bacteriocins (lactacin B, lactacin F and acidocin CH5, nisin and lactocin S (Parada et al., 2007; Vrese and Schrezenmeir, 2008; Zhou et al., 2010; Gao et al., 2019).

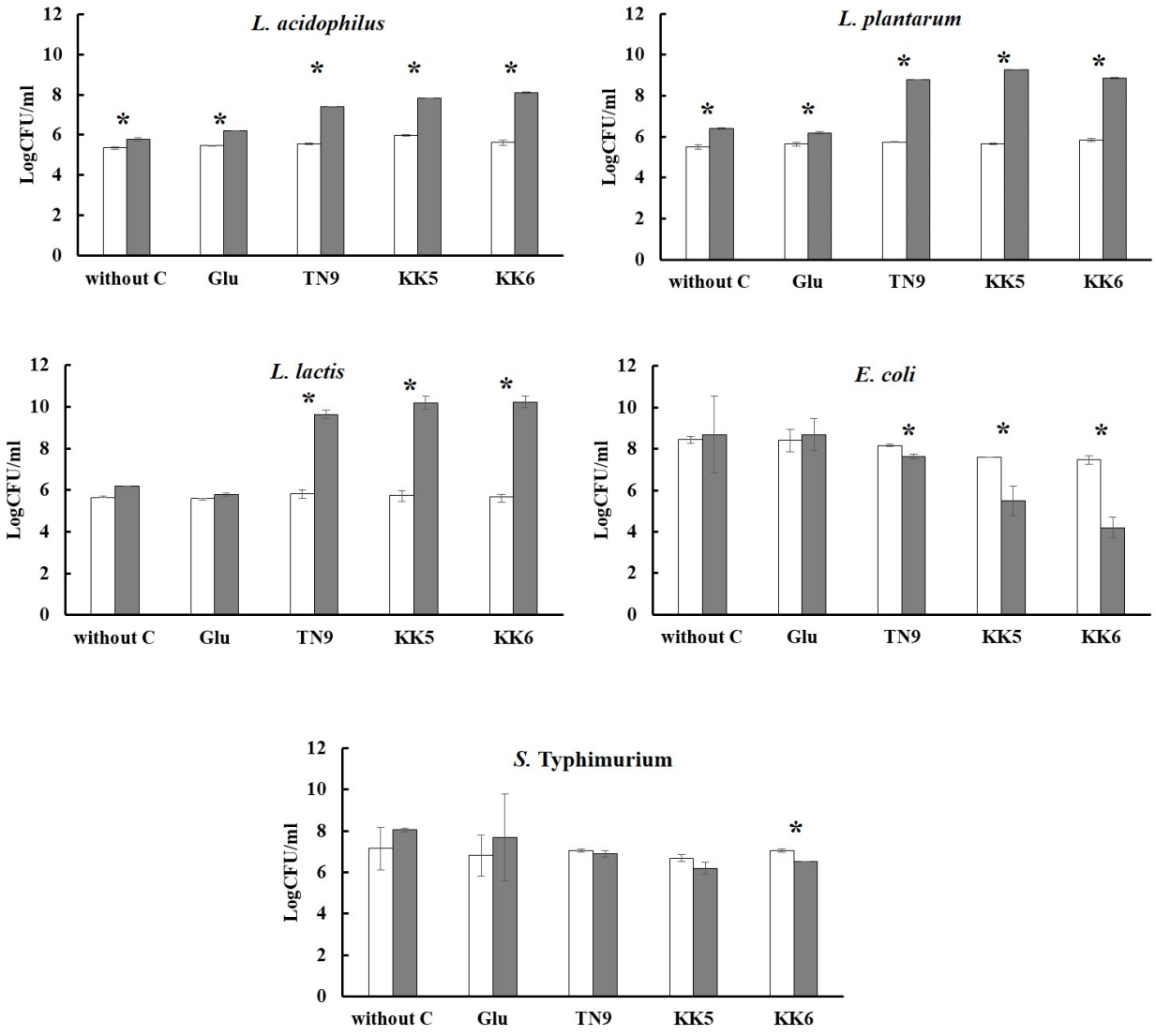


Figure 2. Growth of single tested strains in basal medium supplemented with different carbon source when cultivating for 0 hour (□) and 24 hours (■). The (*) in each experiment indicates significant difference at $p<0.05$.

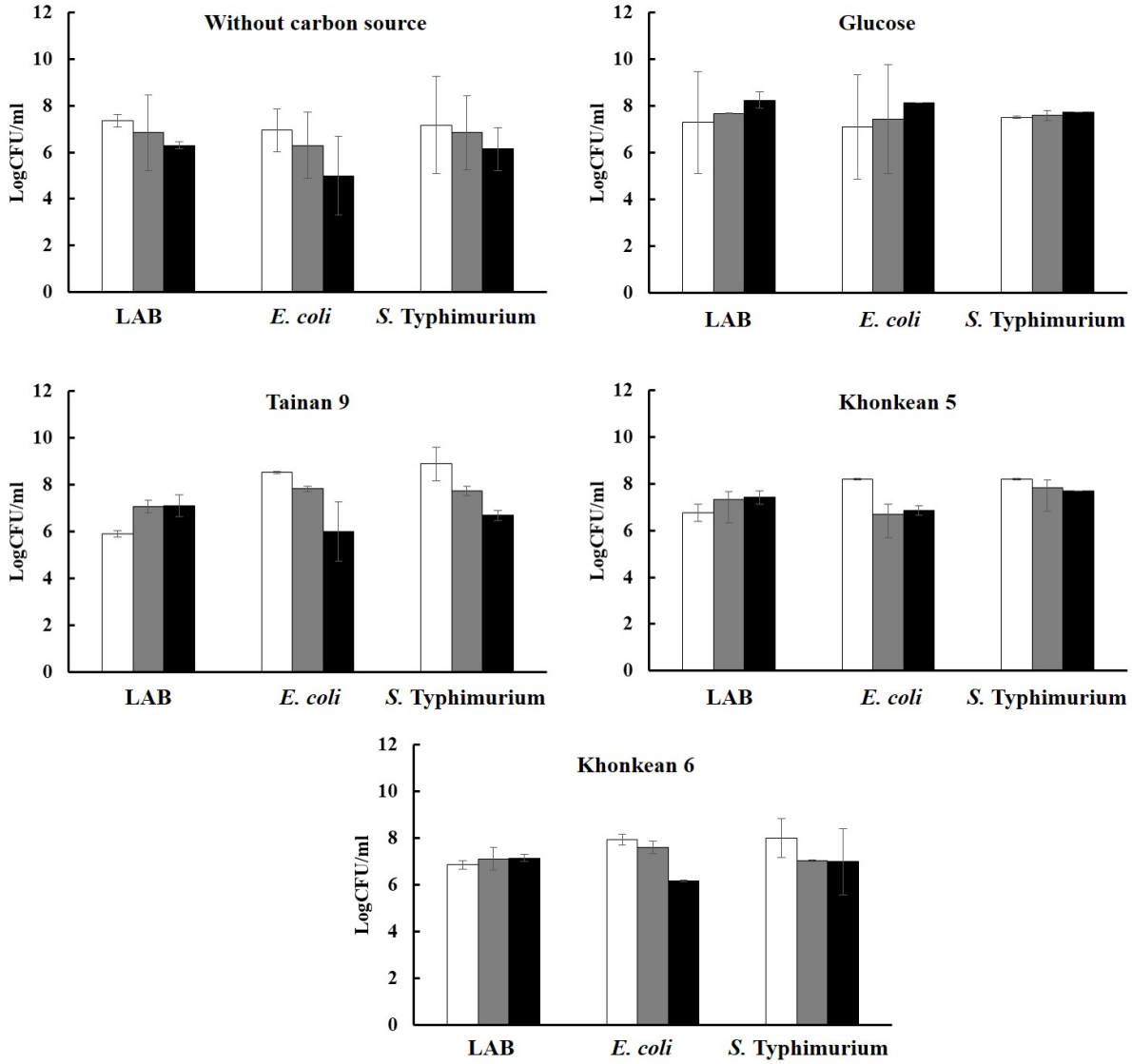


Figure 3. Dynamic of the bacterial population in defined-mixed culture supplemented with different carbon source after 0 hour (□), 12 hours (▒) and 24 hours (■) of cultivation

## 4. CONCLUSION

From all of the results above, the extract prepared from groundnut seeds cultivar Tainan9, Khonkean5 and Khonkean6 showed a potential to be source of an effective prebiotic substance and preliminary exhibited the prebiotic properties by promote growth of probiotic strains; resulting in inhibition of pathogenic growths. Thus consuming of groundnut seed may help to improve the bacterial balance in gastrointestinal tract and receiving of many advantages from grown probiotics. Moreover, synbiotic food containing groundnut RFOs and selective probiotics can be manufactured and promoted as functional foods.

## REFERENCES

Bishi, S.K., L. Kumar, M.C. Dagla, M.K. Mahatma, A.L. Rathnakumar, H.B. Lalwani, and J.B. Misra. 2013. Characterization of Spanish peanut germplasm (*Arachis hypogaea* L.) for sugar profiling and oil quality. *Industrial Crops and Products*, 51: 46-50.

Bishi, S.K., L. Kumar, M.K. Mahatma, N. Khatediya, S.M. Chauhan, and J.B. Misra. 2014. Quality traits of Indian peanut cultivars and their utility as nutritional and functional food. *Food chemistry*, 167: 107-114.

Bryant, R.J., D.R. Rao, and S. Ogutu. 2004. $\alpha$ and $\beta$ galactosidase activities and oligosaccharide content in peanuts. *Plant Foods for Human Nutrition* 58: 213–223.

Chow, J. 2002. Probiotics and prebiotics: a brief overview. *Journal of Renal Nutrition*, 12: 76-86.

Donkor, O.N., A. Henrikson, T. Vasiljevic, and N.P. Shah. 2007. α-galactosidase and proteolytic activities of selected probiotic and dairy cultures in fermented soymilk. *Food Chemistry*, 104: 10-20.

Ekvall, J., R. Stegmark, and M. Nyman. 2007. Optimization of extraction methods for determination of the raffinose family oligosaccharides in leguminous vine peas (*Pisum sativum* L.) and effects of blanching. *Journal of Food Composition and Analysis*, 20: 13-18.

ElSayed, A.I., M.S. Rafudeen, and D. Golldack. 2014. Physiological aspects of raffinose family oligosaccharides in plants: protection against abiotic stress. *Plant Biology* 16: 1-8.

Fredslund, F., A. Hachem, R.J. Larsen, P.G. Sorensen, P.M. Coutinho, L.L. Leggio, and B. Svensson. 2011. Crystal structure of alpha-galactosidase from *Lactobacillus acidophilus* NCFM: insight into tetramer formation and substrate binding. *Journal of Molecular Biology*, 412: 466-480.

Gao, Z., E.B.-M. Daliri, J. Wang, D. Lio, S. Chen, X. Ye, and T. Ding. 2019. Inhibitory effect of lactic acid bacteria on foodborne pathogens: A review. *Journal of Food Protection* 82: 441-453.

Giannoccaro, E., Y. Wang, and P. Chen. 2006. Effects of solvent, temperature, time, solvent-to-sample ratio, sample size and defatting on the extraction of soluble sugars in soybean. *Journal of Food Science*, 71: Published on web.

Gibson, G. R., and M.B. Roberfroid. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *The Journal of nutrition,* 125: 1401-1412.

Hachem, M.A., F. Fredslund, J.M. Andersen, R.J. Larsen, A. Majumder, M. Ejby, G. V. Zanten, S.J. Lahtinen, R. Barrangou, T. Klaenhammer, S. Jacobsen, P.M. Coutinho, L.L. Leggio, B. Svensson. 2012. Raffinose family oligosaccharide utilization by probiotic bacteria: insight into substrate recognition, molecular architecture and diversity of GH36 α–galctosidases. *Biocatalysis and Biotransformation*, 1-10.

Jeong, D-W., J-H. Lee, K.H. Kim, and H.J. Lee. 2006. A food-grade expression/secretion vector for *Lactococcus lactis* that uses an α-galactosidase gene as a selection marker. *Food Microbiology*, 23: 468-475.

Kadlec, P. 2001. Carbohydrate Chemistry. In *Carbohydrates in Grain Legume Seeds: Improving Nutritional Quality and Agronomic Characteristics*, ed. C. L. Hedley, ch.2, 15-60, UK: Biddles Ltd.

Karner, U., T. Peterbauer, V. Raboy, D.A. Jones, C.L. Hedley, and A. Richter. 2004. *myo*-Inositol and sucrose concentrations affect the accumulation of raffinose family oligosaccharides in seeds. *Journal of Experimental Botany*, 55: 1981-1987.

Kumar, V., A. Rani, L. Goyal, A.K. Dixit, J.G. Manjaya, J. Dev, and M. Swamy. 2010. Sucrose and raffinose family oligosaccharides (RFOs) in soybean seeds as influenced by genotype and growing location. *Journal of Agricultural and Food Chemistry*, 58: 5081-5085.

Mahatma, M.K., L. K. Thawait, S. K. Bishi, N. Khatediya, A. L. Rathnakumar, H. B. Lalwani, and J. B. Misra. 2016. Nutritional composition and antioxidant activity of Spanish and Virginia groundnuts (*Arachis hypogaea* L.): a comparative study. *Journal of Food Science and Technology*, 53: 2279-2286.

Martinez-Villaluenga, C., J. Frias, and C. Vidal-Valvrde. 2005. Raffinose family oligosaccharides and sucrose contents in 13 Spanish lupin cultivars. *Food Chemistry,* 91: 645-649.

Mussatto, S.I., and I.M. Mancilha. 2007. Non-digestible oligosaccharides: a review. *Carbohydrate Polymers*, 68: 587-597.

Muzquiz, M., C. Burbano, M.M. Pedrosa, W. Folkman, and K. Gulewicz. 1999. Lupins as a potential source of raffinose family oligosaccharides preparative method for their isolation and purification. *Industrial Crops and Products*, 19: 183-188.

Parada, J.L., C.R. Caron, A.B.P. Medeiros, and C.R. Soccol. 2007. Bacteriocins from lactic acid bacteria: Purification, properties and use as biopreservatives. *Brazilian Archives of Biology and Technology*, 50: 521-542.

Peterbauer, T., J. Mucha, L. Mach, and A. Richter. 2002. Chain elongation of raffinose in pea seeds. *Journal of Chemical Biology*, 227: 194-200.

Saldivar, X., Y.J. Wang, P. Chen, and A. Mauromoustakos. 2010. Effect of blanching and storage conditions on soluble sugar contents in vegetable soybean. *LWT-Food Science and Technology*, 43: 1368-1372.

Schmid, K., and R. Schmitt. 1976. Raffinose metabolism in *Escherichia coli* K12: Purification and properties of a new alpha-galactosidase by specified by a transmissible plasmid. *European Journal of Biochemistry*, 67: 95-104.

Sumarna. 2008. Change of Raffinose and Stachyose in soy milk fermentation by lactic acid bacteria from local fermented foods of Indonesian. *Malaysian Journal of Microbiology*, 4: 26-34

Trugo, L.C., D.C.F. Almeida, and R. Gross. 1988. Oligosaccharide contents in the seeds of cultivated lupins. *Journal of the Science of Food and Agriculture,* 45: 21-24.

Xiaoli, X., Y. Liyi, H. Shuang, L. Wei, S. Yi, M. Hao, Z. Jusong, and Z. Xiaoxiong. 2008. Determination of oligosaccharide contents in 19 cultivars of chickpea (*Cicer arietinum* L.) seeds by high performance liquid chromatography. *Food Chemistry*, 111: 215-219.

Vrese, M., and J. Schrezenmeir. 2008. Probiotics, Prebiotics, and Synbiotics. *Advance in Biochemical Engineering/ Biotechnology*, 111: 1-66.

Wongputtisin, P., R. Ramaraj, Y. Unpaprom, R. Kawaree, and N. Pongtrakul. 2015. Raffinose family oligosaccharides in seed of *Glycine max* cv. Chiang Mai60 and potential source of prebiotic substances. *International Journal of Food Science and Technology*, 5: 1750-1756.

Ziemer, C.J., and G.R. Gibson. 1998. An overview of probiotics, prebiotics and synbiotics in functional food concept: perspectives and future strategies. *International Dairy Journal*, 8: 473-479.

Zhou, X., Y. Wang, J. Yao, and W. Li. 2010. Inhibition ability of probiotic, *Lactococcus lactis*, against *A. hydrophila* and study of its immunostumulatory effect in tilapia (*Oreochromis niloticus*). *International Journal of Engineering, Science and Technology*, 2: 73-80.