PAK. J. FOOD SCI., 27(1), 2017: 1-6
ISSN: 2226-5899

Quality evaluation of ice cream prepared with Sagudana (*Meteroxylon Sagu*) and Sweet Potato (*Ipomoea Batatas*) starch as stabilizing agent

Aysha Sameen[1], Muhammad Faisal Manzoor[2], Nuzhat Huma[1], Amna Sahar[1] and Umair Sattar[1]

[1]National Institute of Food Science and Technology, Faculty of Food, Nutrition and Home Science, University of Agriculture, Faisalabad

[2]Institute of Home and Food Sciences, Faculty of Sciences and Technology, Government College University Faisalabad, Pakistan

Corresponding Author: ayshasameen@uaf.edu.pk

ABSTRACT

Stabilizers are commercially used in ice cream preparation to improve viscosity, air cell distribution, air incorporation, texture and melt down to achieve desirable finished product. In this study, Ice cream samples were prepared using *Ipomoea Batatas* starch and *Meteroxylon Sagu* powder as stabilizers. The sample having commercially available blend (Cremodan) was kept as reference standard. The prepared ice cream was analyzed for physico-chemical (overrun, meltdown, viscosity and standup time) and sensory characteristics at 0.25%, 0.5% and 0.75% concentration of stabilizers. Results showed that ice cream prepared with 0.75% of *Ipomoea Batatas* starch showed significant ($p<0.05$) differences in term of overrun, meltdown, standup time, viscosity compare to *Meteroxylon Sagu* powder and control cremodan. The sensory evaluation findings reported highest score for ice cream samples prepared with 0.75% *Ipomoea Batatas* starch followed by sample containing 0.5% commercial stabilizer and 0.5% *Ipomoea Batatas* starch. These results concluded *Ipomoea Batatas* starch @ 0.75% concentration can be a cost effective and good alternate of traditional stabilizers used for ice cream preparation.

Key words: Sagudana, Sweet potato starch, Stabilizing agent, Ice cream, Overrun

INTRODUCTION

Ice cream is a nutritious, palatable and comparatively less expensive food. No other food attains much popularity and has a fascinating appeal as ice cream (Goff, 2002). A compositional range for ice cream components which is used in mixture is milk fat 9-16%, MSNF 9-11%, corn syrup solids 3-6%, sucrose 10-12%, stabilizer and emulsifier 0-0.5%, total solid 37-44% and water 56-63% (Goff, 1997). Stabilizers are valuable constituent of ice cream mix. Stabilizers slightly change the acidity of the mix, enhance the viscosity, whipping time and surface tension. The stabilizer like gelatin, starch or pectin are used in milk to improve the following ice cream characteristics such as appearance, mouth feel, viscosity and texture. The quantity of stabilizer which is used depends on the quality and kind compulsory to produce the desirable stabilizing effect in the final product.

Sweet potato starch alone account for 60-70% of calories intake of human (Chandan, 2006). Sweet potato is highly nutritive and good source of vitamins (A and C), minerals (iron, potassium, calcium) and fiber. It is essential for immune function, vision, skin and bone health (Khan *et al.* 2008). Beside its nutritive value, starch is a very versatile raw material it has many application in food, feed, pharmaceutical, paper, textile and cosmetic industries. In the food industry, starch used as a thickener to increase the solid content, to consolidate the mass of food as a stabilizer (Burrel, 2003). In developing countries, the production of sweet potato about 95% of the world. In Pakistan, sweet potato production is 11,951 tons according to Ozturk *et al.* (2012). In food industry application of starch in bakery product, beverages, dessert, sauces, dressings, meat and dairy product (Sajilata *et al.* 2006).

Sagu powder (*Meteroxylon sagu*) is principally used as a thickening and stabilizing agent in the food industry. Sagu palm which is mostly grown in the islands of Malaysia and Indonesia and contributed almost 70%of all sagu production. Sagu is rich in carbohydrate. Nutritionally, low in fat and high in dietary fiber and minerals such as iron and calcium sagu is healthful fat replacer (Walter and Sam, 2002). Presently in ice cream industries imported and costly stabilizers blends are using. So, the purpose of present research to discover the best locally available natural and halal stabilizers which can be used as substitute to

costly imported stabilizers and can be used in small and large ice cream industries.

MATERIALS AND METHODS

Procurement of raw material

The fresh milk, milk cream and other ingredients such as sugar, skim milk powder, stabilizer (*sagudana*, sweet potato starch) artificial flavor and food grade color (FD&C yellow, 5) was purchased from the local market.

Extraction of starch

Sweet potato starch was extracted without chemical by following the method described by Oladebeye *et al.* (2009).

Analysis of sweet potato and Sagudana powder

Ash content, moisture, and crude protein were measured by the method described in AOAC (2000). pH was determined by using pH meter (WTW series pH-720). Water holding capacity, swelling power and solubility of starch were estimated by method described by Garg and Jana (2011). Viscosity of starch was evaluated by using Brookfield DV-E viscometer by following method reported by Mweta (2009).

Ice cream preparation

The ingredients like stabilizers, sugars, and skimmed milk powder were weighed and mixed with liquid milk and milk cream through constant mechanical stirring. Sweet potato starch and sagudana powder were used in different concentrations (0.25, 0.5 and 0.75%) as shown in Table (1). The mixture was pasteurized for 30 min at 72^0C and then homogenization was done with electric homogenizer (U/MIN 7000 Type B-1 Elek tromischer Made in Germany). After the homogenization, ageing was perfumed for 5 hours at 4 ^{0}C. The mixture was further subjected at low temperature of –1 to $–9^0$C along with whipping of air for ice cream (Schmidt, 2004). The ice cream physicochemical and sensory evaluation was filled in disposable cups (100 mL).

Physico-chemical and sensory evaluation

The viscosity was estimated by Brookfield DV-E viscometer according to the method of Sevim et al. (2001). Meltdown and standup time were determined according to method of Bhandari (2001). While calculation of overrun was done by the method described Varnam and Sutherland (1994). Sensory evaluation was carried out by panel of 5 judges using 9-point hedonic scale (Larmond, 1977).

Statistical analysis

Data statistical analyzed for the effects of the factors on standup time, viscosity, over-run, meltdown time and sensory evaluation was performed by CRD using SPSS software to determine the level of significance (Steel *et al.* 1997). The factors were: type of stabilizers (sweet potato starch, sagudana powder) and concentration (0.25, 0.5 and 0.75%).

RESULTS AND DISCUSSION

Analysis of starch and powder

Sagudana powder and sweet potato starch was subject to different analysis. All the analyses were performed in triplicate and mean values are presented in Table 2. For sweet potato values for pH (5.38-5.60), swelling power (10.05-10.28%), solubility (3.30-3.33%), water holding capacity (82.70-83.54%), moisture (10.5-10.6%), ash (0.71-0.75), protein (0.30-0.33%) and viscosity (7550-7562cp). However, for sagudana for pH (5.30-5.50), swelling power (25.1-27.2%), solubility (0.55-1.0%), water holding capacity (78.2-79.5%), moisture (10.2-10.5%), ash (0.70-0.74), protein (0.35-0.38%) and viscosity (3550-3605cp). pH, water holding capacity, solubility and viscosity of sweet potato starch were greater than sagudana powder whereas swelling power of sagudana was higher than sweet potato. The results obtained in present study are in line with the findings of Mweta *et al.* (2009).

Analysis of ice cream

Mix viscosity

The effect of different concentrations of sweet potato starch and sagudana powder on mix viscosity of frozen ice cream are shown in Table 3 and Figure 1. All concentrations of sweet potato starch and sagudana powder showed significant ($P<0.05$) effect on viscosity of ice cream among all treatments. The linear increase of viscosity was observed with increasing the concentration of starch sweet potato and sagudana. The highest value for viscosity was achieved for sweet potato starch at concentration of 0.75% a compare to the controlled. The factors which can affect the viscosity include temperature, type, concentration, state of stabilizer and fat globule size. Viscosity can also provide mouth feel and flavor to the ice cream (Hematyar *et al.* 2012). If milk protein and fat are increase then viscosity is also increase but whereas in all the samples these components were equal so viscosity was increase only due to differences in type and quantity of stabilizers (Tarkash & Yadolah, 2005). High water holding capacity of a stabilizer effected the rheological properties of mix (Guinard *et al.* 1994) so undoubtedly increase in viscosity is depending upon the quantity of stabilizers (Rosalina *et al.* 2004).

Table 1. Different concentrations of sweet potato starch and sagudana powder in ice cream preparation

Treatments	Control	Sweet potato starch (%)	Sagudana (%)	Total
T_0	0.5	0	0	0.5
T_1	0	0.25	0	0.25
T_2	0	0	0.25	0.25
T_3	0	0.5	0	0.5
T_4	0	0	0.5	0.5
T_5	0	0.75	0	0.75
T_6	0	0	0.75	0.75

Table 2. Physicochemical analysis of sweet potato starch and sagudana powder

Physicochemical analysis	Sweet potato starch	Sagudana powder
pH	5.38±0.10	5.30±0.05
Swelling power %	10.05±0.2	27.2±0.1
Solubility %	3.30±0.3	0.55±0.2
Water holding capacity %	82.70±0.5	78.2±0.5
Moisture %	10.5±0.2	10.2±0.3
Ash %	0.71±0.04	0.70±0.03
Protein %	0.30±0.03	0.35±0.02
Viscosity (cp)	7550±40	3550±30

Table 3. Comparison of means for physico-chemical analysis according to different stabilizers

Treatments	T_0	T_1	T_2	T_3	T_4	T_5	T_6
Viscosity (cP)	2950±20	2360±21	1950±18	2646.7±16	2126.7±15	3053.3±16	2443.3±15
Meltdown (mL/10min)	19.93±0.15	24.20±0.12	30.20±0.13	21.20±0.11	27.56±0.16	18.96±0.14	26.50±0.13
Overrun (%)	52.58±0.27	43.74±0.62	38.49±0.63	46.87±0.60	40.70±0.75	52.64±0.94	43.24±0.67
Standup time (min.)	11.20±0.10	9.45±0.05	7.21±0.11	10.2±0.15	8.01±0.12	12.10±0.12	9.05±0.13

Table 4. Comparison of means for sensory characteristics as influenced by treatments

Treatments	T_0	T_1	T_2	T_3	T_4	T_5	T_6
Flavor	7.4±0.54	6±0.83	5.4±0.54	6.8±0.70	5.8±0.89	7.8±0.83	6.2±0.81
Taste	7.6±0.54	6.2±0.70	5.5±0.50	6.9±0.81	5.9±0.53	7.9±0.70	6.4±0.85
Appearance	7.7±0.83	6.2±0.83	5.6±0.52	7.2±0.83	6.4±0.54	8.0±0.70	6.8±0.80
Body/Texture	7.6±0.54	5.8±0.80	5.4±0.50	6.7±0.70	5.8±0.89	7.8±0.83	6.4±0.80
Overall acceptability	7.8±0.83	6.0±0.70	5.8±0.80	7.0±0.83	6.2±0.85	8.0±0.70	6.6±0.70

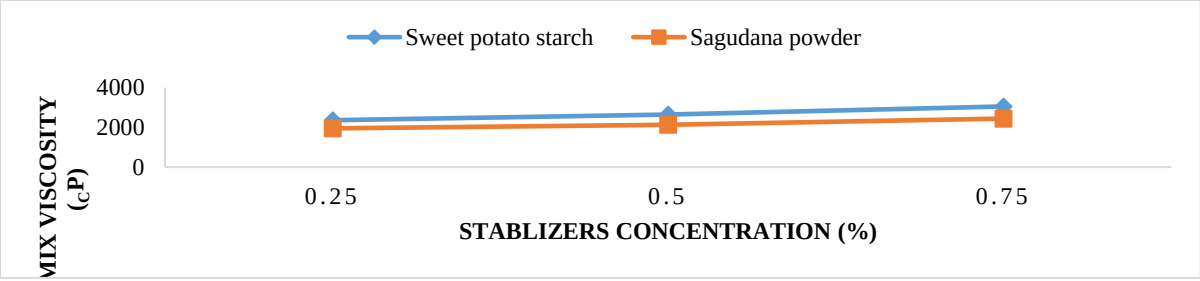

Fig. 1. Mix viscosity of ice cream prepared using sweet potato starch and sagudana powder stabilizer

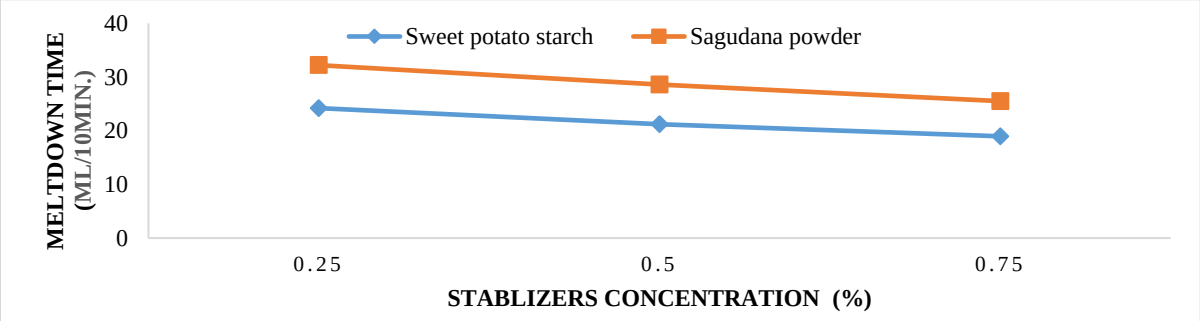

Fig. 2. Meltdown time (ml/10min.) of ice cream prepared using sweet potato starch and sagudana powder

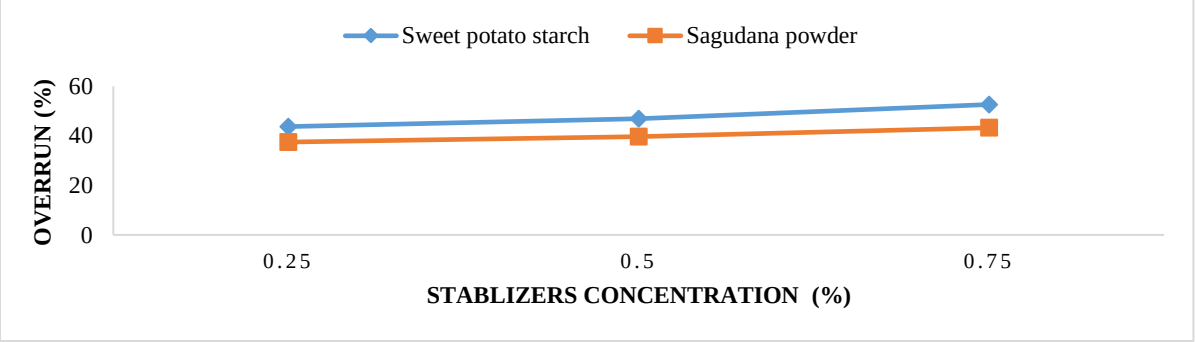

Fig 3. Overrun (%) of ice cream prepared using sweet potato starch and sagudana powder stabilizers

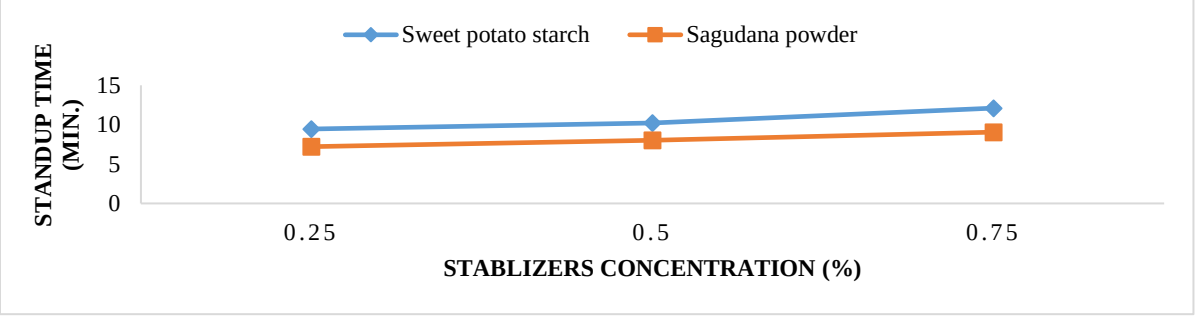

Fig. 4. Standup time (min.) of ice cream prepared using sweet potato starch and sagudana powder stabilizers

Melt down

The melt down effect of stabilizer in ice cream are shown Table 3 and Figure 2. It was observed that all concentrations of sweet potato starch and sagudana powder have significant ($P<0.05$) melt down effects among all treatments. The lowest meltdown was observed for sweet potato starch at 0.75% concentration and highest meltdown was observed for sagudana powder 0.25% concentration. Melting

resistance is significantly affected by type and concentration of stabilizers. The melt down of ice cream was affected by its composition and additives and many other, such as the amount of air incorporated, ice crystals nature and fat globules network formed during freezing (Koxholt *et al.* 2001). Ice creams have high overrun began to melt slowly and whereas ice creams have low overrun melted quickly. The primary cause of quick melting is low freezing point and then environmental conditions (Marshall & Arbuckle, 1996). The melting of the ice is controlled by the outside temperature and heat transfer. Homogenization improves melt down property of ice cream (Goff, 2001). Ice cream has desirable melting quality when the melted ice cream is very similar in characteristics to that of the original mix (Bhandari, 2001). Results of study describe a correlation between results of over-run and meltdown time as over-run enhances meltdown.

Over run

The results showed that among all treatments different concentration of sweet potato starch and sagudana powder have significant ($P<0.05$) effect on overrun. The highest value was observed for sweet potato starch at concentration of 0.75% shown in Table 3 and Figure 3. Stabilizer type and quantity had a significant effect on over-run. As overrun decreases, ice crystals and air cells become smaller in size. However, there is the counter balancing effect of weakening of the structure because of thinning of the unfrozen material among the air cells and ice crystals (Marshall *et al.* 2003). Potter and Hotchkiss (1995) described the shrinkage in ice cream due to collapse of weakened films of mix, causing the ice cream to lose volume. Due to loss of air the shrinkage was reported by Rothwell (1993). Amount of air in ice cream is directly related to the over run, is important because incorporation of air effect the product quality and profits. The less incorporation of air produce soggy and too much produce a fluffy ice cream (Igoe, 1979).

Standup time

The period which elapsed before the first drop of melted ice cream fell was noted for each sample. Addition of sweet potato starch and sagudana powder at different concentrations showed significant ($P<0.05$) effect on the stand-up time of ice cream mix. The highest standup time in minutes was also observed in ice cream samples having 0.75% concentration of sweet potato starch, while the lowest standup time was observed in ice cream sample having 0.25% sagudana powder concentration shown in Table 3 and Figure 4. Stabilizer type and quantity had a significant effect on the standup time of ice cream mix. Investigation show that significantly difference in standup time by increasing the concentration of stabilizers. The period which elapsed before the first drop of melted ice cream fell was noted for each sample. Ice cream with high melting quality begins to show definite melting within 10-15 minutes when placed at room temperature. The standup time for normal ice cream is 13 min. at 20 °C (Marshall and Arbuckle, 1996).

Sensory evaluation of ice cream

Samples were organoleptically evaluated for appearance, taste, flavor, body/texture and overall acceptability, by the panel of 5 judges. All the sensory parameters of sweet potato starch and sagudana powder were non-significantly affected by ice cream samples except sweet potato starch at 0.75% concentration significantly effected as compare to controlled (Table 4). The ice cream sample get highest awarded by judge's panel containing 0.75% sweet potato starch followed by the ice cream containing 0.5% sweet potato starch. While ice cream containing sagudana powder stabilizers got the lowest scores.

Conclusions

Stabilizer that could be recommended for this product was sweet potato starch at 0.75% for best viscosity, meltdown, over-run and sensory characteristics. It is concluded that ice cream made with locally available sweet potato starch as stabilizer showed the comparable results from commercially used imported stabilizer. Therefore, by using locally made and available stabilizers blends, the production cost can be minimized and foreign exchange can be saved. The ethical concerns have resulted in a global interest for Halal and natural stabilizer.

Acknowledgment

All the authors do not have any affiliations with in any organization for any financial interest in the subject materials discussed in this manuscript. There is no conflict of interest among authors regarding the submission of this research work.

REFERENCES

1. Bhandari, V. 2001. Ice Cream Manufacture and Technology. Tata McGraw Hill Pub. Co. Ltd. New Delhi.
2. Burrell, M.M. 2003. Starch: the need for improved quality or quality – an overview. J. Exp. Bot. 55: 451- 456.
3. Chandan, R.C. 2006. History and consumption trends. In manufacturing yoghurt and fermented milks. Blackwell publishing, Ames, I.A.pp.3-16.
4. Garg, S. and A.K. Jana. 2011. Characterization and evaluation of acylated starch with different acyl groups and degrees of substitution. Carbs Poly. 83: 1623-1630.

5. Goff, H. D. 2002. Formation and stabilization of structure in ice-cream and related products. Current Opinion Coll Interface Sci. 7: 432–437.
6. Goff, H.D. 1997. Colloidal aspects of ice cream–A review. International Dairy Journal, 7: 363–373.
7. Goff, H.D. 2001. Controlling ice-cream structure by examining fat: Protein interactions. Aust. J. Dairy Technol. 55:78-81.
8. Guinard, J.X., C. Liitle, C. Marty and T.R Palchakz. 1994. Effect of Sugar and Acid on the Acceptability of Frozen Yogurt to a Student Population. J. Dairy Sci. 77: 1232-1238.
9. Hematyar, N., A.M. Samarin, H. Poorazarang and A. H. Elhamirad. 2012. Effect of Gums on Yogurt Characteristics. World App. Sci. J. 20 (5): 661-665.
10. Igoe, S.R. 1979. Compositions for stabilizing soft serve and hard frozen yogurt. United States Patent 4178390.
11. Khan, K., S. Rehman, M.A. Khan, F. Anwar and S. Bhadar. 2008. Physical and chemical quality appraisal of commercial yoghurt brands sold at Lahore. ARPN J. Agri. Biol. Sci. 3: 14-20.
12. Koxholt, M.M.R., B. Eisenmann and J. Hinrichs. 2001. Effect of the fat globule sizes on the meltdown of ice cream. Journal of Dairy Science, 84(1):31-7.
13. Larmond, E. 1977. Laboratory Methods for Sensory Evaluation of Foods. Canada Department of Agriculture, Pub. No. 1637.
14. Marshall, R.T. and W. S. Arbuckle. 1996. Ice cream. New York: Chapman and Hall.
15. Marshall, R.T., H. D. Goff and R.W Hartel. 2003. Ice cream. 6th Ed., New York: Kluwer Academic/Plenum Publishers.
16. Mweta, D.E. 2009. Physicochemical, functional and structural properties of native malawian cocoyam and Sweet potato starches. Ph.D. diss., Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein, South Africa.
17. Official Methods of Analysis of AOAC International. 2000. Official Methods of Analysis. 17th Ed. AOAC International, Gaithersburg, MD, USA.
18. Oladebeye, A.O., A.A. Oshodi and A.A. Oladebeye. 2009. Physicochemical properties of starches of Sweet potato (Ipomeabatata) and red cocoyam (Colocasiaesculenta) cormels. Pak. J. Nutri. 8: 313-315.
19. Ozturk, G., F.N. Azeri and Z. Yildirim. 2012. Field performance of in vitro Sweet potato (Ipomoea batatas) plantlets derived from seed stocks. Turk. J. Field Crops. 17: 14-20.
20. Potter, N.N. and J.H Hotchkiss. 1995. Food Science 5th Edition. Chapman & Hall, New York. Public Health Association, Washington, DC.
21. Rosalina, P., Sofian, Hartel and J.H. Richard. 2004. Effects of overrun on structural and physical characteristics of ice cream. Int. Dairy J. 14(3): 255-262.
22. Rothwell, J. 1993. Defect of Ice Cream arising during distribution and storage. Ice Cream and Frozen Confection. 45(1): 4-5.
23. Sajilata, M.G., R. S. Singhal and P.R. Kulkarni. 2006. A review-comprehensive reviews in Food Science and Food Safety. J. Food Agri. Environ. 5:1-17.
24. Schmidt, K.A. (2004). Dairy: Ice Cream, in Food Processing: Principles and Applications (eds J. S. Smith and Y. H. Hui), Blackwell Publishing, Ames, Iowa, USA
25. Sevim, K. and A.R. Tekin. 2011. The effect of salep content on the rheological characteristics of a typical ice-cream mix. J. Food Eng. 47: 59-62.
26. Steel, R.G.D., J.H. Torrie and D.A. Dicky. 1997. Principles and Procedures of Statistics. A Biometrical Approach, (3rd ed.) McGraw Hill Book Co. Inc., New York.
27. Tarkashvand, and Yadolah. 2005. Ice cream. First print. Ata publisher, Tehran (in persion).
28. Varnam, A.H. and J.P. Sutherland. (1994). Milk and Milk Products. Food Products Series, Chapman and Hall, London.
29. Walter, A. E. and C. Sam. 2002. Fruits of Oceania.ACIAR Monograph
85; Paris, Canberra/IRD.

PAK. J. FOOD SCI., 27(1), 2017: 7-14
ISSN: 2226-5899

Preparation, characterization and rheological properties of vitamin E enriched nanoemulsion

Hafiz Rizwan Sharif [1], Mian Kamran Sharif [2*] and Fang Zhong[1]

[1]State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi 214122, Jiangsu, People's Republic of China.

[2]National Institute of Food Science & Technology, Faculty of Food, Nutrition & Home Sciences, University of Agriculture, Faisalabad 38040, Pakistan.

[*] Corresponding author: mks@uaf.edu.pk

ABSTRACT

Vitamin E (VE) is an essential micronutrient, consumed by peoples to reduces occurrence of various diseases. Higher chemical instability and poor water solubility of VE demands its encapsulation. Present study reports the encapsulation of VE into octenyl succinic anhydride modified starch (OSA-MS) Purity Gum Ultra (PGU) based nanoemulsions. Influence of different concentrations (0, 2, 4 & 6%) of VE on nanoemulsions size, polydispersity index (PDI), zeta potential (ZP) and rheological properties were determined. Results showed that as concentrations of VE increased, a gradual increase in size (179 to 208 nm) and PDI (0.09 to 0.12) was observed, whereas the trend for ZP (– 34.5 to – 17.9) showed opposite pattern. Size distribution results showed broadness with the increasing VE concentrations. Rheological parameters showed that all prepared emulsions had shear thinning properties (flow behavior index: $n < 1$) and a direct correlation was found between concentrations of VE and viscosity of emulsions. Furthermore, emulsion containing 4% VE was selected (on the bases of size (197.1 nm), ZP (– 31.7) and viscosity (1.62 Pa.s)) and subjected to 4 weeks storage at 25 ± 2 °C for the evaluation of physical stability. There was a gradual increase in size of nanoemulsion was recorded with respect to the storage time, however, the increase was quite small which did not cause any physical instability of the emulsion. Consequently, nanoemulsion containing 4% VE stabilized by PGU could be used for the development of functional foods and beverages.

Keywords: Vitamin E, nanoemulsions, Purity Gum Ultra, physical stability, rheology

INTRODUCTION

Vitamin E (VE) is a fat-soluble compound that naturally presents in eight different isomers and among all, α-tocopherol is the most naturally abundant and biologically active form of VE in humans. Consumption of adequate levels of α-tocopherol links to prevent various kinds of health disorders due to its biological activity and antioxidant properties (Traber *et al.*, 2008; Cordero *et al.*, 2010; Saberi *et al.*, 2013). Recently, trend of functional foods development has got a considerable attention by the food industry to improve the health and well-being of consumers. However, lipophilic bioactives are sensitive to deterioration and oxidation which reduce their activity and efficacy. In addition, low water solubility hampers their use in aqueous food systems and reduces their delivery in edible form. Therefore, it is beneficial to encapsulate lipophilic bioactives into an appropriate cost effective delivery system, which improves physico chemical stability and bioavailability of the encapsulated bioactives (Sharif *et al.*, 2017a).

Nanotechnology is a science of miniature that involves manufacturing, characterization and manipulation of materials which have a size range at the nanometer scale (Peters *et al.*, 2016). Commonly used encapsulation methods in the food industry includes high pressure homogenization, sonication, microfluidization, spray drying, spray cooling/chilling, fluidized-bed coating, freeze drying, coacervation, extrusion, co-crystallization, inclusion complexation, solvent evaporation, immobilization, phase inversion, and liposomes, etc. (Sharif *et al.*, 2017b).

Nanoemulsions are thermodynamically unstable colloidal dispersions having size (diameter) below 200 nm (Ostertag *et al.*, 2012). The smaller size offers clarity to the nanoemulsions and consequently their application into transparent food and beverage products (Mayer *et al.*, 2013a). For the preparation of nanoemulsions, two immiscible liquids (oil and water) are mixed (dispersed) to form a homogeneous dispersion (emulsion) with the help of an emulsifier/surfactant. An emulsifier must have both

hydrophobic and hydrophilic groups (amphiphilic nature) to lower the surface tension between two liquids. Two basic approaches, low-energy (spontaneous emulsification, phase inversion temperature and emulsion inversion point) and high-energy (ultrasonication, microfluidization and high pressure homogenization) are commonly employed to facilitate the emulsification process (Mayer *et al.*, 2013b). The stability of nanoemulsions depend upon many factors including type of emulsifier, concentration of emulsifier, type of lipid phase, method of emulsification and storage conditions.

Octenyl succinic anhydride modified starches (OSA-MS) have been used as food additive (E1450) for more than fifty years (Sweedman *et al.*, 2013). Applications of OSA-MS in emulsification, stabilization, encapsulation, film formation and gel production have been documented by various researchers (Mao *et al.*, 2009; Abbas *et al.*, 2014; Liang *et al.*, 2013; Hategekimana *et al.*, 2015a; Majeed *et al.*, 2016a; Sharif *et al.*, 2017a). Purity Gum Ultra (PGU) is a newly developed waxy maize OSA-MS. It has good emulsification properties and needs at lower concentration. Abbas *et al.* (2015) prepared curcumin based stable nanoemulsions using PGU as emulsifier. Recently, Sharif *et al.* (2017a) utilized PGU for the co-encapsulation of flax seed oil and eugenol. The objectives of the present work were to formulate a stable delivery system enriched with VE and stabilized with PGU, which could be further used for development of functional foods and beverages.

MATERIALS AND METHODS

Materials

Vitamin E (α-tocopherol) with ≥ 97.0% purity was purchased from Sigma-Aldrich (St. Louis, MO). Cold pressed flax seed oil was obtained from TA Foods Ltd. (Yorkton, Saskatchewan, Canada), where the major fatty acids were: oleic acid (~ 17.87%), linoleic acid (~ 15.87%) and linolenic acid (~ 57.0%). Purity Gum Ultra (PGU) was obtained from National starch, USA. All other chemicals used in this study were of analytical grade. The water used in all experiments was double distilled.

Preparation of oil in water (O/W) VE enriched nanoemulsion

Nanoemulsions were prepared by following the method of Sharif *et al.* (2017a) with some modifications. Briefly, an aqueous solution of PGU was prepared by dispersing the dried powder at concentration of 2% (w/v) into double distilled water at room temperature. The solution was kept stirring overnight to ensure complete hydration. Flax seed oil was used as carrier oil and different concentrations of VE (0, 2, 4 & 6% w/w) were dissolved in it. After preparation of oil and aqueous phases, coarse emulsions were prepared by homogenizing the oil (10% w/w) and aqueous phases (90% w/w) with a high-speed homogenizer (Ultra-Turrax, Germany) at 18,000 rpm for 5 min at room temperature. Finally, the coarse emulsions were then passed through a microfluidizer (Model 101, Microfluidics, Newton, MA) at 110 MPa pressure with 6 processing cycles to obtain fine droplets.

Determination of size and polydispersity index (PDI) of emulsion

After preparation of emulsions, the size was recorded as mean droplet diameter (MDD) through dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments, Malvern, U.K.). To minimize multiple light scattering effects, emulsions were diluted with double distilled water at 1:100 and agitated well. A refractive index values used for flax seed oil and water were 1.45 and 1.33, respectively. Size measurements of fresh and stored emulsions were carried out by selecting 11 numbers of runs and expressed in nm, and the PDI was calculated by cumulant analysis. All measurements were carried out in triplicate and were conducted at room temperature.

Determination of Zeta Potential (ZP)

The zeta potential of emulsions was determined using Zetasizer (Zetasizer Nano ZS, Malvern Instruments, Malvern, U.K. at room temperature. Emulsions were diluted with double distilled water at 1:100 and placed in clear disposable zeta cell. The number of runs was set from 10 (minimum) to 100 (maximum) and ZP was calculated from the electrophoretic mobility using the Smoluchowski equation. All measurements were made in triplicate and values were expressed in mV.

Determination of Rheological Properties

The rheological properties of VE enriched nanoemulsions were calculated by following the method of Sharif *et al.* (2017a) using the Discovery Hybrid Rheometer (DHR-2, TA-Instruments, New Castle, DE, USA) with a cone and plate geometry (cone diameter = 40 mm, angle = 2^{o}, gap = 48 µm). For each measurement, ~ 1 mL of the emulsion sample was loaded between cone and plate fixtures. The viscosity of VE enriched nanoemulsions was measured by a steady state flow program with the

shear rate ranging from 1 s^{-1} to 1000 s^{-1}. Experimental flow curves were fitted to a power law model

$$\eta=K\gamma^{n-1} \quad (1)$$

Where, η is the viscosity (Pa·s), K is the consistency index (Pa·s^n), γ is the shear rate (s^{-1}), and n is the flow behavior index. The temperature was maintained at 25 °C during all measurements.

Statistical Analysis

The data obtained were further analyzed and presented as mean ± standard deviation. One-way ANOVA was used and Duncan's multiple-range test was performed at $P < 0.05$ to compare the mean values. All statistical analysis was performed using SPSS Statistics (software version 19.0) according to the procedure defined by Steel et al. (1997).

RESULTS AND DISCUSSION

size and PDI of enriched nanoemulsions

Emulsions were characterized for their mean droplet diameter (MDD). Results presented in Fig. 1a revealed that with the increasing VE concentrations, a continuous increase in MDD of emulsions was observed. Emulsions containing VE up to 4% showed nanometric size (d < 200 nm), whereas at 6% VE concentration emulsion crossed the nanometric range (208.0 ± 2.03). Hategekimana *et al.* (2015b) also reported an increasing trend in emulsion droplet size with the increasing VE concentrations in carrier oil phase. The possible reason of rise in size of emulsions with the increasing VE concentrations could be the increase in viscosity of the dispersed phase due to highly viscous nature of VE. Consequently, higher energy inputs are needed to disrupt the particles into fine emulsions. Polydispersity index (PDI) is an important parameter which shows distribution of particles in a colloidal system and narrow range of PDI favors the system stability. Mostly, PDI values below 0.3 are considered as good, which leads to ensure system stability. The results of PDI presented in Fig. 1a depicted that emulsions were stable. However, a continuous increase in PDI was also noted with the increasing VE concentrations in system. Furthermore, results of size distributions (Fig. 1b) also indicated narrow size distributions at lower VE concentrations. Emulsions containing 6% VE displayed the wider size distribution as compared to the other samples.

Zeta Potential (ZP) of VE Enriched Nanoemulsions

Determination of ZP is a very critical factor, as it influences the stability of dispersed oil droplets. ZP values raging from very high positive to very high negative are considered better. Higher ZP means better the electrostatic repulsion between the dispersed particles, which favors long term stability (Dinda *et al.,* 2013). Results of ZP ranged from – 17.9 ± 0.31 to – 34.5 ± 0.25 mV (Fig. 2a). Concentrations of VE were also found influential and with the increasing VE contents a decline in ZP values was observed, which was significantly ($p > 0.05$) lower at 6% VE. Negative ZP values were due to the presence of negative charge (carboxylic groups) on OSA-MS, which appears during esterification process (Nilsson *et al.,* 2007). The present results are in accordance with our previous studies, which showed higher negative ZP values of emulsions stabilized by OSA-MS (Liang *et al.,* 2012; Abbas *et al.,* 2014; Majeed *et al.,* 2016b; Sharif *et al.,* 2017a).

Rheology Properties of VE Enriched Nanoemulsions

Rheological properties of emulsions are very important as these links to the stability and final product application. Emulsions viscosity was evaluated under the influence of shear rate. A direct correlation was found between viscosity and VE concentrations in emulsions (Fig. 2b). A significant ($p > 0.05$) increase in viscosity (3.48 ± 0.05 Pa.s) was noted at 6% VE concentrations and a direct relation was noted between viscosity and the size of emulsions. Emulsion containing 6% VE showed highest values of viscosity and size (above 200 nm). Size, ZP and rheological studies showed that nanoemulsions with good physical characteristics can be prepared using up to 4% VE. Data obtained was also subjected to the power law model and found that all emulsions showed a shear thinning behavior under shear rate. There exist three value ranges for n (flow behavior index): $n < 1$ for a shear-thinning fluid, $n = 1$ for a Newtonian fluid, and $n > 1$ for a shear-thickening fluid. All emulsions showed less than 1 n value. The similar kind of trend was previously reported in medium chain triglycerides-pepper mint oil and flax seed oil-eugenol based nanoemulsions stabilized by OSA-MS, respectively (Liang *et al.,* 2012; Sharif *et al.,* 2017a).

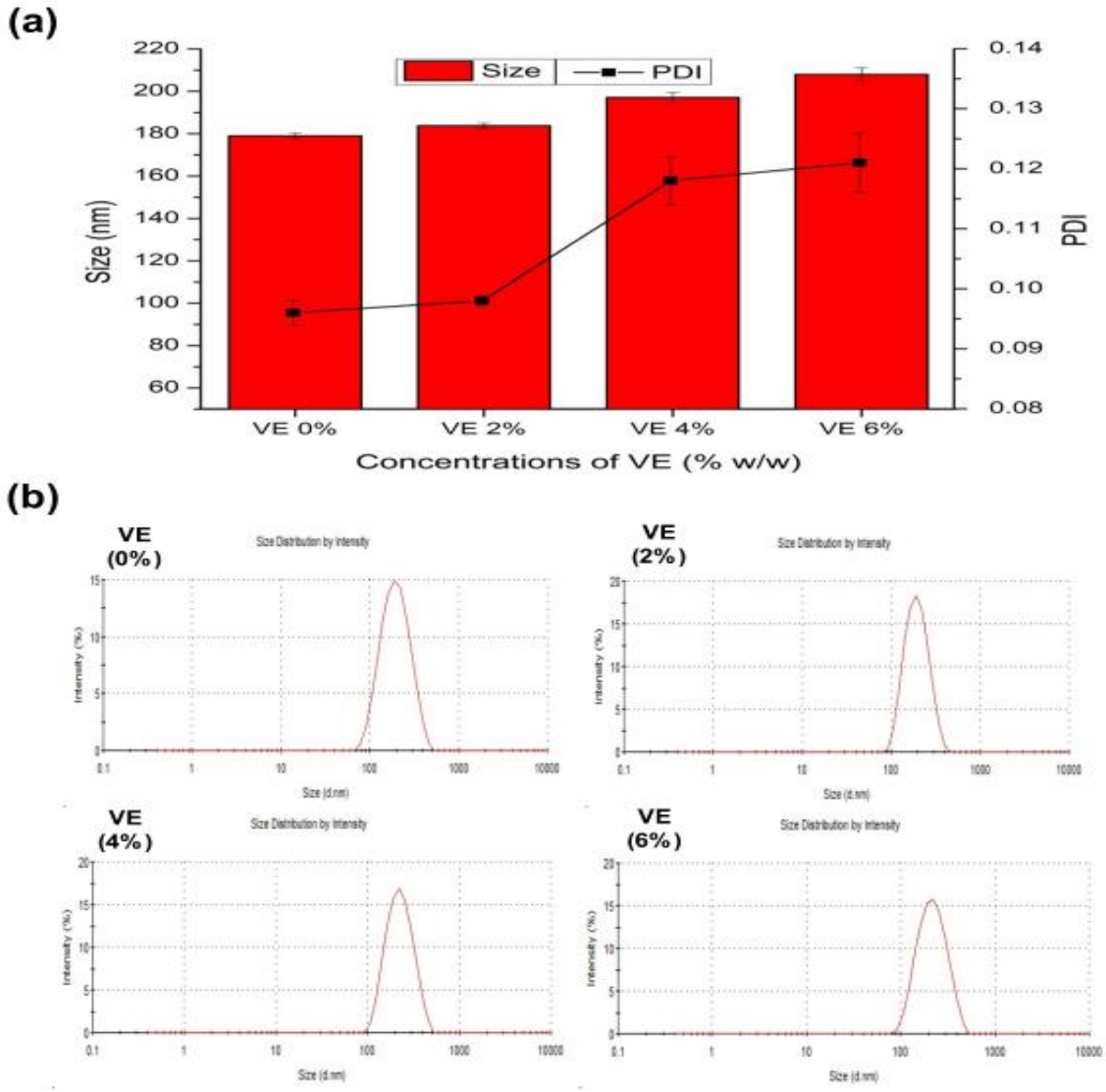

Figure 1. Effect of different concentrations (0, 2, 4 & 6% w/w) of vitamin E (VE) on **(a)** size, PDI **(b)** and size distribution of nanoemulsions

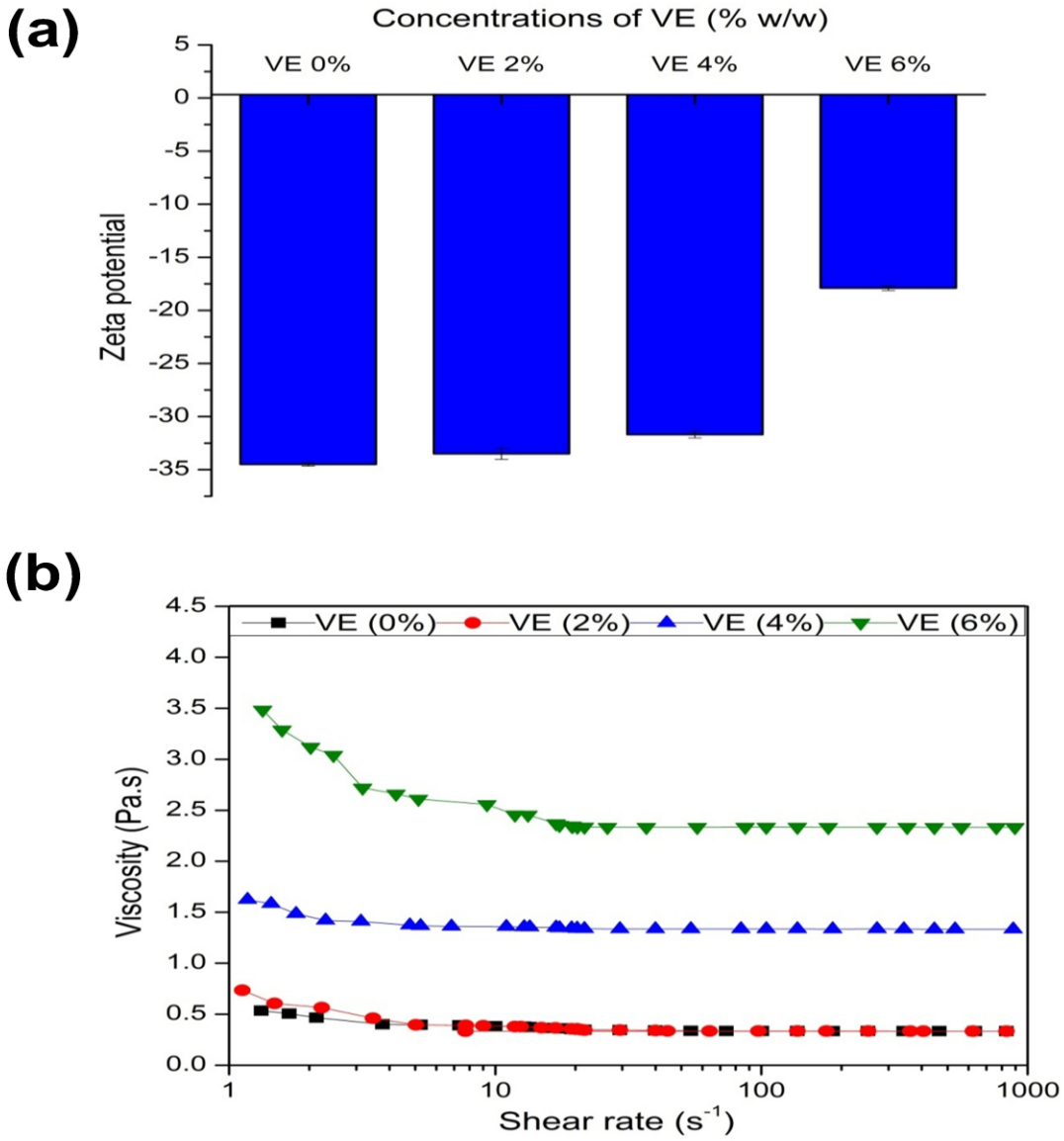

Figure 2. Effect of different concentrations (0, 2, 4 & 6% w/w) of vitamin E (VE) on **(a)** zeta potential (ZP) and **(b)** viscosity of nanoemulsions

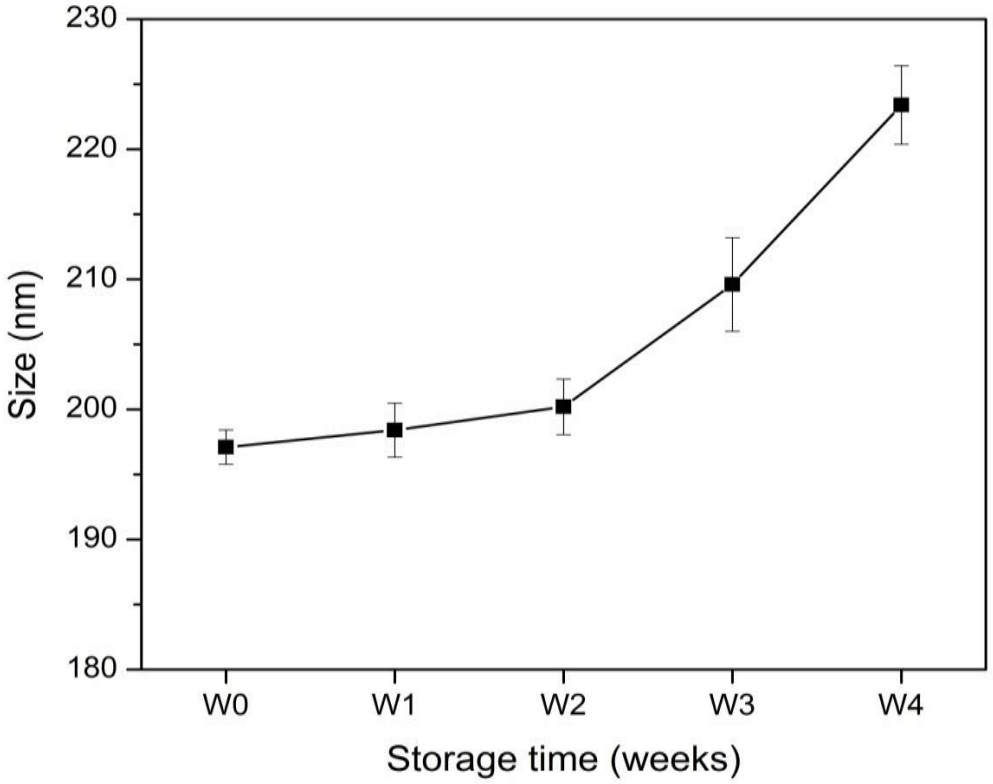

Figure 3. Effect of storage time (4 weeks at 25 °C) on size of nanoemulsion containing 4% (w/w) vitamin E

Storage Stability

On the bases of optimization of VE concentrations in emulsions, nanoemulsion containing 4% VE was selected and further stored for 4 weeks at room temperature (25 ± 2 °C). During storage there was no physical separation/layering observed. Further the emulsion was characterized for change in size during storage. There was a gradual increase in size with respect to storage time was observed (Fig. 3). Initially, for the first 2 weeks the increase in size was non-significant ($p < 0.05$) and emulsion did not cross the nano metric size limit ($d<200$ nm). After 2 weeks, the increase in size was significant ($p>0.05$) and maximum size (223.4 ± 3.71 nm) was noted at 4^{th} weeks of storage. Overall, there was ≈ 26 nm increase in size was noted during 4 weeks of storage. Such small increase in size showed that emulsion containing 4% VE was quite stable. Previously, Liang *et al.* (2013); Abbas *et al.* (2014); Majeed *et al.*, (2016b) and Sharif *et al.* (2017a) reported ≈ 20-30, 20-25, 15-50 and 10-40 nm increase in size of nanoemulsions during storage, respectively.

CONCLUSIONS

Emulsions containing different concentrations (0, 2, 4 & 6%) of VE were formulated using PGU as emulsifier. Size distribution results showed that up to 4% VE can be successfully encapsulated and at 6% VE concentrations emulsion crossed the nano metric size ($d < 200$ nm) range. All emulsions showed negative ZP and below 1 n (flow behavior index) values. Nanoemulsion containing 4% VE showed physical stability during 4 weeks storage at room temperature. The present findings will be helpful to formulate VE enriched nanoemulsions and development of functional foods and beverages.

Acknowledgement

This study was sponsored by Chinese Scholarship Council under Chinese Government.

REFERENCES

1. Abbas, S., E. Karangwa, M. Bashari, K. Hayat, X. Hong, H.R. Sharif and X. Zhang. 2015. Fabrication of polymeric nanocapsules from curcumin-loaded nanoemulsion templates by self-assembly. Ultrason. Sonochem. 23: 81-92.

2. Abbas, S., M. Bashari, W. Akhtar, W.W. Li and X. Zhang. 2014. Process optimization of ultrasound-assisted curcumin nanoemulsions stabilized by OSA-modified starch. Ultrason. Sonochem. 21(4): 1265-1274.

3. Cordero, Z., D. Drogan, C. Weikert and H. Boeing. 2010. Vitamin E and risk of cardiovascular diseases: a review of epidemiologic and clinical trial studies. Crit. Rev. Food Sci. Nutr. 50(5): 420-440.

4. Dinda, A., I. Biswal, P. Chowdhury and R. Mohapatra. 2013. Formulation development and evaluation of paclitaxel loaded solid lipid nanoparticles using glyceryl monostearate. J. Applied Pharm. Sci. 3(8): 133-138.

5. Hategekimana, J., M.V.M. Chamba, C.F. Shoemaker, H. Majeed and F. Zhong. 2015b. Vitamin E nanoemulsions by emulsion phase inversion: Effect of environmental stress and long-term storage on stability and degradation in different carrier oil types. Colloids Surf. A: Physicochem. Eng. Aspects. 483: 70-80.

6. Hategekimana, J., K.G. Masamba, J. Ma and F. Zhong. 2015a. Encapsulation of vitamin E: Effect of physicochemical properties of wall material on retention and stability. Carbohydr. Polym. 124, 172-179.

7. Liang, R., C.F. Shoemaker, X. Yang, F. Zhong and Q. Huang. 2013. Stability and bioaccessibility of β-Carotene in nanoemulsions stabilized by modified starches. J. Agric. Food Chem. 61(6): 1249-1257.

8. Liang, R., S. Xu, C.F. Shoemaker, Y. Li, F. Zhong and Q. Huang. 2012. Physical and antimicrobial properties of peppermint oil nanoemulsions. J. Agric. Food Chem. 60(30): 7548-7555.

9. Majeed, H., F. Liu, J. Hategekimana, H.R. Sharif, J. Qi, B. Ali, Y.-Y. Bian, J. Ma, W. Yokoyama and F. Zhong. 2016b. Bactericidal action mechanism of negatively charged food grade clove oil nanoemulsions. Food Chem. 197: 75-83.

10. Majeed, H., J. Antoniou, J. Hategekimana, H.R. Sharif, J. Haider, F. Liu, B. Ali, L. Rong, J. Ma and F. Zhong. 2016a. Influence of carrier oil type, particle size on in vitro lipid digestion and eugenol release in emulsion and nanoemulsions. Food Hydrocolloid. 52: 415-422.

11. Mao, L., X. Duoxia, Y. Jia, Y. Fang, G. Yanxiang and Z. Jian. 2009. Effects of small and large molecule emulsifiers on the characteristics of β-carotene nanoemulsions prepared by high pressure homogenization. Food Technol. Biotechnol. 47(3): 336-342.

12. Mayer, S., J. Weiss and D.J. McClements. 2013a. Behavior of vitamin E acetate delivery systems under simulated gastrointestinal conditions: Lipid digestion and bioaccessibility of low-energy nanoemulsions. J. Colloid Interface Sci. 404: 215-222.

13. Mayer, S., J. Weiss and D.J. McClements. 2013b. Vitamin E-enriched nanoemulsions formed by emulsion phase inversion: Factors influencing droplet size and stability. J. Colloid Interface Sci. 402: 122-130.

14. Nilsson, L. and B. Bergenståhl. 2007. Adsorption of hydrophobically modified anionic starch at oppositely charged oil/water interfaces. J. Colloid Interface Sci. 308 (2): 508-513.

15. Ostertag, F., J. Weiss and D.J. McClements. 2012. Low-energy formation of edible nanoemulsions: Factors influencing droplet size produced by emulsion phase inversion. J. Colloid Interface Sci. 388: 95-102.

16. Peters, R.J.B., H. Bouwmeester, S. Gottardo, V. Amenta, M. Arena, P. Brandhoff, H.J.P. Marvin, A. Mech, *et al.*, 2016. Nanomaterials for products and application in agriculture, feed and food. Trends Food Sci. Technol. 54: 155-164.

17. Saberi A.H., F. Yuan and D.J. McClements. 2013. Fabrication of vitamin E-enriched nanoemulsions: Factors affecting particle size using spontaneous emulsification. J. Colloid Interface Sci. 391: 95-102.

18. Sharif, H.R., P.A. Williams, M.K. Sharif, M.A. Khan, H. Majeed, W. Safdar, M. Shamoon, M. Shoaib, J. Haider and F. Zhong. 2017a. Influence of OSA-starch on the physico chemical characteristics of flax seed oil-eugenol nanoemulsions. Food Hydrocol. 66: 365-377.

19. Sharif, H.R., P.A. Williams, M.K. Sharif, S. Abbas, H. Majeed, K.G. Masamba, W. Safdar and F. Zhong. 2017b. Current progress in the utilization of native and modified legume proteins as emulsifiers and encapsulants-A review. Food Hydrocolloid. http://dx.doi.org/10.1016/j.foodhyd.2017.01.002.

20. Steel, R.G.D., J.H. Torrie and D.A. Dicky. 1997. Principles and procedures of Statistics. A

Biometrical Approach. 3rd ed. McGraw Hill Book Co. Inc., New York.

21. Sweedman, M.C., M.J. Tizzotti, C. Schäfer and R.G. Gilbert. 2013. Structure and physicochemical properties of octenyl succinic anhydride modified starches: A review. Carbohydr. Polym. 92(1): 905-920.

22. Traber, M.G., B. Frei and J.S. Beckman. 2008. Vitamin E revisited: do new data validate benefits for chronic disease prevention? Curr. Opin. Lipidol. 19(1): 30-38.

PAK. J. FOOD SCI., 27(1), 2017: 15-25
ISSN: 2226-5899

Influence of antioxidants from date and fenugreek seeds on shelf life of coconut biscuits

Hira Khan[1], Salim-ur-Rehman[1*], Imran Pasha[1], Khalil-ur-Rehman[2], Muhammad Ismail Siddique[3] and Aafia Khalid[3]
[1]National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan
[2]Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan
[3]Department of Food Science and Technology, Lyallpur Campus, University of Sargodha, Faisalabad, Pakistan

***Corresponding author:** drsalim_rehman@yahoo.com

ABSTRACT

Shelf stability of coconut biscuits is a major problem during storage due to their high content of unsaturated fatty acids. The objective of this study was to evaluate comparative antioxidants potential of date and fenugreek seeds phenolics used to enhance the shelf life of coconut biscuits. Coconut biscuits were prepared by adding seed extracts at different percentages into coconut biscuits and stored for 60 days at an ambient temperature. Results revealed that moisture, fat, protein, ash, fiber and NFE of biscuits were affected non-significantly as a result of antioxidants. The effects on TPC, DPPH, peroxide value, physical and sensory properties were evaluated during the storage study of 60 days. The effect of antioxidants during storage on TPC, DPPH and water activity was highly significant, while on peroxide value, color, and texture, the effect was non-significant. Sensory evaluation based on color, flavor, taste, texture and overall acceptability was also affected non-significantly. All treatments remained acceptable during 60 days of storage. However, coconut biscuits with 0.03% date seeds phenolics (T_3) gained maximum scores during storage.

Key words: Antioxidant, shelf life, date seeds, fenugreek seeds, phenolics

INTRODUCTION

Biscuits are a common food eaten by a wide range of the population, because of their varied taste, durability and relatively low cost commodity. Because of the competition in the market and increased demand for natural, healthy and functional products, various attempts have been made to improve the nutritional value and functionality of biscuits by changing their composition. Such effects are usually fulfilled by increasing the ratios of dietary fiber as well as antioxidant contents in the biscuits (Tyagi *et al*., 2007). Shelf life of coconut biscuits is a major problem occurring during preservation. They have short shelf life due to their high content of unsaturated fatty acids.

Use of antioxidants reduces the occurrence of rancidity in processed foods. Use of antioxidants is a secular concept. Synthetic antioxidants show higher efficiency to inhibit oxidation of fats even at low concentrations that is why they are used extensively in the bakery products. The commonly used artificial antioxidants are Tertiary butyl hydroxy quinone (TBHQ), Butylated hydroxyl anisole (BHA), Butylated hydroxy toluene (BHT) and Gallates etc. They can easily be incorporated into food products and available at low price. However, now-a-days, the consumers demand has been increased for the use of natural antioxidants in their foods (Nanditha and Prabhasankar, 2008). Presently, natural antioxidants become the essential food additives due to their exclusive properties of increasing shelf life without damaging the sensory and nutritional quality of foods.

The date palm (*Phoenix dactylifera*) is a tropical and subtropical tree. It belongs to the family Palmae (*Arecaceae*). It is the oldest cultivated plant in the Arabian region. It is cultivated mainly in the hot arid regions. Saudi Arabia is the world largest producers of dates. The date fruits are conventionally used in the preparation of some products such as fermented products and date pastes. Their seeds are either used as animal feed or gone waste (Al-Hooti *et al*., 2002). By one estimate, the seed represents an average of 10 % of the dates. Date seeds contain 3-7% moisture, 3-6% protein, 5-13% fat and 0.8-1% ash. They are rich in dietary fiber and polyphenol contents. Dietary fiber has health potential benefits in diabetes and cancer prevention. Chemical compounds like phenolic and flavonoids are included in polyphenols. Their antioxidant properties play important role in inactivation of chain reactions of lipid free radicals, preventing hydro-peroxide conversion into reactive ox radicals, chelating redox-active metal ions and from their anti-inflammatory properties (Joseph *et*

al., 2005). Phenolic and flavonoid compounds are directly related to the antioxidant contents; both have reducing power and radical scavenging properties. Concentration of epicatechin is relatively low in date seeds because catechin is directly related with reducing the radical scavenging power.

The varieties of some date seeds are used as a functional ingredient. Those varieties show the lowest amount of fat with the highest antioxidant capacity. The seeds which contain the maximum levels of phenols and flavonoids can show the maximum antioxidant effect of polyphenols by minimizing fat intake. Polyphenols play an important role in the prevention of diseases like cardiovascular diseases, diabetes and cancer. This is said to be the cause of the powerful antioxidant and anticancer activities of phenolic compounds and flavonoids (Platat *et al.,* 2014). Fenugreek (*Trigonella foenumgraecum*) is an annual herb. It belongs to the family *Leguminosae*. It is known as 'Methi' in Hindi. It is used as spice in Pakistan, Asian, African and European countries. Its leaves are consumed as vegetable and its seeds are commonly used in food preparations as a spice. It has strong aroma and flavor and is used to stimulate the digestive processes and have many other therapeutic and nutritional properties. Fenugreek seeds contain 7-9% moisture, 3-4% ash, 6-8 % fat, 7-8% fiber and 28- 30% protein. Clinical and experimental studies have shown the anti-diabetic and anti-atherosclerotic effects. Studies showed that fenugreek seeds are rich in bioactive compounds such as antioxidants. Its seeds contain antioxidants and polyphenols that inhibit oxidative hemolysis and also lipid peroxidation that are induced in human erythrocytes by H_2O_2 (Kaviarasan *et al*., 2004).

Reduction in biomarkers of oxidative damage may be achieved by adding fenugreek seed powder in feed. Many results and evidence revealed that many health advantages can be obtained from food sources that are rich in antioxidant nutrients (Aruoma, 1998). These polyphenols are mainly present in seeds, roots, nuts, leaves and other parts of the plants. These showed great value in the area of medicine and food chemistry because of their antioxidant properties including suitable biological effects. Seeds of fenugreek are ground to have many medicinal traits like carminative, demulcent, tonic, astringent, restorative, emollient and antifungal properties that are used to cure mouth ulcers, stomach irritation, dried lips, lower blood sugar concentration as it is evident from Iranian tradition (Hajimehdipoor *et al.,* 2010). The alkaloids, flavonoids and saponins of fenugreek seeds have anti-nociceptive, anti-diabetic and hypocholesterolaemic properties (Acharya *et al.,* 2006). However, scientific research shows its more use in functional foods and neutraceuticals (Kumar and Maliakel, 2008). Considering the beneficial effect of these, this project was designed to enhance the shelf life of coconut biscuits by adding date and fenugreek seed extracts in different concentrations during storage at ambient temperature.

MATERIALS AND METHODS

Procurement of raw materials

Date seeds, fenugreek seeds, coconut powder, flour, sugar, oil and baking powder were procured from local market. Analytical grade chemicals were purchased from Sigma-Aldrich, Germany. The research was conducted in the laboratories of National Institute of Food Science and Technology, University of Agriculture, Faisalabad–Pakistan.

Chemical analysis of date and fenugreek seeds

Date and fenugreek seeds were analyzed for moisture, crude protein, crude fat, crude fiber, ash content and nitrogen free extract according to the method as described in AOAC (2006).

Table 1. Treatments plan

Treatments	Antioxidant extract (%)
T_0	Control without antioxidants
T_1	0.03%
T_2	0.015%
T_3	0.03%
T_4	0.045%
T_5	0.015%
T_6	0.03%
T_7	0.045%

T_0= without antioxidant
T_1= BHT (butylated hydroxy toluene)
T_2, T_3, T_4 = Date seed extract
T_5, T_6, T_7 = Fenugreek seed extract

Product development

Coconut biscuits were prepared with different levels of date and fenugreek seeds extract according to treatment plan as given in Table (1). Coconut biscuits were prepared according to the method given in AACC (2003) with certain modifications. The recipe followed was flour (200g), shortening (150g), sugar (125g), coconut powder (50g), baking powder (2g) and egg (1). Creaming was done with mixer in 10min by mixing shortening and sugar. Then eggs was added and mixing was done for few minutes. Then flour, coconut powder and baking powder were added and mixed. This mixture was thoroughly mixed for 5min. After that molding in to biscuits, baking was done at 180ºC for 10min. After cooling at ambient temperature, coconut biscuits were packed in polythene bags and stored for 60 days at ambient temperature. These were analyzed for their antioxidant activity, physical and sensory analyses at 0, 15, 30, 45 and 60 days interval.

Proximate analysis of coconut biscuits

Coconut biscuits were analyzed for moisture, crude protein, crude fat, crude fiber, NFE and ash contents according to the method as described in AOAC (2006).

Phenolic content

Total phenolic contents (TPC) in coconut biscuits were quantified at different storage intervals by using Folin-Ciocalteu method (Iqbal *et al.*, 2005) that was based on the reduction of phosphotungstic acid to phosphotungstic blue and as result of it absorbance increased due to rise in number of aromatic phenolic groups. For the purpose, 200µL of sample were separately added to test tube containing 1mL of Folin-Ciocalteu's reagent, 2mL of 7.5% sodium carbonate solution and volume was made up to 7mL with distilled water. The absorbance of the resulting blue color was measured at 765nm on UV/visible light Spectrophotometer. Total polyphenols were estimated and values were verbalized as gallic acid equivalent (mg Gallic acid/g). Total phenolic compounds of each sample in Gallic acid equivalents (GAE) were calculated by following formula:

$$C=c\times V/ m$$

C = Total phenolic contents (mg/g extract, in GAE)

c = Concentration of Gallic acid (mg/mL)

V = Volume of extract (mL)

m = Weight of sample (g)

Free radical scavenging activity (DPPH assay)

DPPH (1,1-diphenyl-1-picrylhydrazyl) is a stable and highly colored oxidizing radical that result in formation of a yellow colored hydrazine (DPPH-H) associated with abstraction of free hydrogen atoms from phenolic antioxidants. Protocol of Tadhani and Subhash (2007) was followed to determine DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity of coconut biscuits at different storage intervals. 4mg of DPPH were dissolved in 100 mL methanol and 2 mL of this solution were added to 50µL sample extract. The mixture was shaken vigorously and allowed to stand at room temperature in the dark. Then the absorbance was measured at 515 nm. The radical scavenging percentage was calculated using the following equation. The free radical-scavenging activity of each sample is presented as percentage reduction in DPPH due to given amount of each extract.

Reduction of absorbance (%) = [(AB – AA) / AB] × 100

AB = Absorbance of blank sample at t = 0min; AA = Absorbance of tested extract solution at t = 15min

Peroxide value

1g of sample was weighed into a clean dried boiled tube and 1g of powdered potassium iodide and 20 mL of solvent mixture were added. Tube was placed in boiled water so that the liquid boiled within 30sec and boiling allowed vigorously for not more than 30sec. Those contents transferred quickly to a conical flask that contained 20mL of 5% potassium iodide solution. The tube was washed twice with 25mL water each time and collected in a conical flask. It was titrated against N/500 sodium thiosulphate solution till yellow color disappeared. 0.5mL of starch solution was added, shaked vigorously and titrated carefully till the blue color just disappeared. A blank was also set at the same time (Sadasivam and Manickam. 2008).

Per oxide value (milli equivalent peroxide/ kg sample) = $\frac{S\times N\times 1000}{\text{Sample (g)}}$

S = mL $Na_2S_2O_3$

N = normality of $Na_2S_2O_3$

Physical analysis

Color

The color of coconut biscuits was determined at different storage intervals according to method as described by Rocha and Morais (2003) with help of colorimeter. The colorimeter was calibrated by using standards (54 CTn for dark and 151 CTn for light). The color of coconut biscuits was determined by placing the sample under the photocell.

Texture

Texture of coconut biscuits was analyzed at different storage intervals according to Manisha *et al*. (2012) by using a textural analyzer (Model. TA-XT2, Stable Microsystems, Surrey, UK) with a 5kg load cell. The Textural skilled program version 4 was used for data analysis. Textural determinations were made by using a 75mm compression Platen (P/75) for a compression test. Samples were positioned centrally with the right angles to the direction of forces. Once a trigger force of 100g was achieved, the compression platen proceeded down toward the coconut biscuits and rapid rise in the force was observed. During this stage, the sample is deformed under the applied force but there was no apparent breakdown of the product. As the compression distance increased, small peaks were found on the graph profile; each peak indicating a compressive failure of the sample. This stage ended abruptly when the test completed and was indicated as a large decrease in force. The greater the distance that was occured, the greater was the ability to withstand compression without sample breakage.

Water activity

Water activity of coconut biscuits was determined at different storage intervals as described by Sidhu *et al*, (1999) at different storage intervals by using an electronic Hygro palm water activity. (Rotronic Hygro palm *Aw*, Series no. 601089738) Hygro palm having 9v Rechargeable battery, it is a portable temperature and humidity indicator. Probe was attached to the remote unit that shows measurements by showing on screen. The ground sample was placed in the plastic jar and then Hygro palm probe was inserted in it. Then enter button was pressed to access the function of the remote unit. The up and down buttons were used to navigate the function menu, the enter key was pressed to confirm the settings. The display had shown the water activity (0-1), along with temperature (^{o}C) and time (s).

Sensory evaluation

Sensory evaluation (color, flavor, taste, texture and overall acceptability) was performed at 0, 15, 30, 45 and 60 days' storage intervals according to the method of Lawless and Heymann (2010).

Statistical analysis

The obtained results were subjected to statistical analysis to estimate the significance by using 2 factor factorial and Duncan multiple range tests.

RESULTS AND DISCUSSION

Chemical composition of date and fenugreek seeds

Proximate composition of the date seeds illustrates that it contains moisture, protein, fat, fiber, ash and NFE as 8.16±0.10, 5.54±0.50, 9.76±0.27, 17.36±0.50, 1.02±0.02 and 58.16±0.36%, respectively. Al-Farsi *et al.* (2007) described the proximate composition of date seeds and their reported composition was 3.1–7.1% moisture, 5.0–13.2% fat, 2.3–6.4% protein and 0.9–1.8% ash. Juhaimi *et al.* (2012) performed chemical analysis on seven date seed varieties and concluded that proximate composition of date seeds was found between the ranges as moisture contents 7.87-9.01%, crude fat contents 4.68-7.96%, crude protein contents 3.71-5.47%, crude fiber17.07-23.46% and ash contents 1.03-1.26%. Proximate composition of the fenugreek seeds illustrates that it contains moisture, protein, fat, fiber, ash and NFE as 10.53±0.19, 24.5±1.75, 5.66±0.19, 3.79±0.12, 3.72±0.11 and 52.26±1.74%, respectively. The results of the proximate composition were resembled to the findings of Naidu *et al*. (2011), as they illustrated the percentages of moisture, protein, fat, fiber and ash in fenugreek seeds were 11.44±0.01, 27.57±0.09, 6.71±0.01, 3.6±0.14 and 3.9±0.14%, correspondingly. Similarly, Kaur *et al*. (2011) described proximate profile of fenugreek seeds and it was found in accordance with the results of present research.

Proximate analysis of coconut biscuits

Effect of different treatments on the proximate composition of coconut biscuits is shown in Table 2. It is concluded from the results that composition was not significantly affected by the addition of date and fenugreek seed extracts. The highest moisture was observed in T_2 (5.91±0.09%) while moisture level of T_0 was found the lowest (4.97±0.85%). The highest ash content was observed in T_4 (1.76±0.05%) while

ash content of T_0 was found the lowest (1.50±0.1%). The highest fat was observed in T_4 (40±3.60%) while fat content in T_0 was the lowest **(**34.6±4.58%). The highest protein content was observed in T_5 (7.80±0.05%) while protein content of T_0 was the lowest (7.27±0.48%). The highest fiber content was observed in T_7 (4.82±0.17%), whereas the fiber content of T_0 was the lowest 4.55±0.20%. The highest NFE value was observed in T_0 (47.03±3.08%) while NFE value of T_4 was the lowest (39.72±3.65%). These results are found in accordance with the findings of Dhankhar (2013).

Phenolic content

The results showed that there was a significant effect of treatments and storage periods on total phenolic contents (mg GAE/100g) of biscuits. (Tables 3, 4). The TPC of the treatments determined in this study range from 43.89±4.34 to 92.08±6.02 (mg GAE/100g). The maximum content of TPC (92.08±6.02mg GAE/100g) was observed in T_4 followed by T_7 (85.65±5.03mg GAE/100g), T_1 (85.46±6.44mg GAE/100g), T_3 (81.46±5.16mg GAE/100g), T_6 (75.93±4.54mg GAE/100g), T_2 (72.08±5.04mg GAE/100g) and T_5 (67.55±4.53mg GAE/100g). However, the minimum TPC (43.89±4.34mg GAE/100g) was found in T_o. During the storage period, the declining trend in total phenolic contents was observed. The range between 0 day and 60 days was found to be 80.16±14.50-68.99±14.15mg GAE/100g. These results are found agree with the findings of Ajila *et al.* (2010) and Verardo *et al.* (2011) who reported that storage of biscuits showed decrease in phenolic compounds. This could be due to the oxidative changes occurring during storage of biscuits.

Free radical scavenging activity (DPPH assay)

DPPH is test applied to quantify the antioxidant activity of a food product. The results showed that there was a significant effect of treatments and storage periods on antioxidant activity of biscuits (Tables 3, 4). The range for the antioxidant activity was found from 19.62±2.30 to 52.33±5.10%. Highest antioxidant activity (52.33±5.10%) was observed in T_4 followed by T_1 (49.61±3.87%), T_7 (48.05±4.23%), T_3 (46.56±4.84%), T_6 (44.49±4.16%), T_2 (36.39±4.35%) and T_5 (34.21±4.07%). However, the lowest antioxidant activity (19.62±2.30%) was found in T_o. During storage study, it was observed that there was a gradual decrease in the antioxidant activity. The minimum antioxidant activity was observed at 60th day, while highest was found at 0 day of storage 36.35±9.53% and 46.73±11.16% respectively. Magda *et al.* (2008) reported that the antioxidant activity decreased in stored food products.

Peroxide value

Analysis of peroxide value may be applied to determine the development of rancidity during storage period. The lowest peroxide value means, minimum oxidative rancidity occurred during storage (Kilcast and Subramaniam, 2000). Analysis showed that there was a significant effect of treatments on peroxide value of biscuits while storage showed a non-significant effect on peroxide value as presented in Tables 3, 4. The range for the peroxide value was found from 0.027±0.001 to 0.096±0.004mEq/Kg. The lowest peroxide value 0.027±0.001mEq/Kg was observed T_4 followed by T_7 (0.030±0.001mEq/Kg), T_1 (0.040±0.002mEq/Kg), T_3 (0.050±0.002mEq/Kg), T_6 (0.05±0.002mEq/Kg), T_5 (0.061±0.003 mEq/Kg) and T_2 (0.072±0.003mEq/Kg). However, the highest peroxide value (0.096±0.004 mEq/Kg) was found in T_o. During storage study, it was observed that there was a gradual increase in the peroxide value. The minimum peroxide value (0.0525±0.021mEq/Kg) was observed at 0 day while the highest (0.0554±0.021mEq/Kg) was found at 60th day of storage. Priya and Ramaswamy (2016) reported the peroxide value of cookies developed by using coconut products. They concluded that the peroxide values of cookies were 0.02±0.01 and on storage the value increased up to 0.03±0.01. On comparing the value of storage at 0 day and 56th day, no significant difference was observed. Seevaratnam *et al.* (2012) and Aruna (2000) conducted a study on the formulated biscuits. Their studies also indicated that no rancidity development was observed during 60 days of storage.

Physical analysis

Results for the physical analysis of coconut biscuits are presented in the Tables 5, 6. The color measurement is mainly performed with CIELAB color system and its attributes are L*, a* and b* where L* is the indicator of lightness-darkness, a* indicates greenish to reddish tonality, whereas b* represents bluish to yellowish tonality. Color of coconut biscuits showed significant differences in L* value owing to difference in treatments and non-significant decrease in storage. Similarly, it also

Table 2. Effect of different treatment on the means of proximate composition of coconut biscuits

Treatments	Moisture	Ash	Fat	Protein	Fiber	NFE
T_0	4.9767±0.85	1.50±0.1	34.6±4.58	7.27±0.48	4.55±0.20	47.03±3.18
T_1	5.5167±0.14	1.53±0.05	37±2	7.65±0.10	4.70±0.29	43.59±2.12
T_2	5.9133±0.09	1.66±0.15	36±2.51	7.40±0.48	4.73±0.27	44.29±5.26
T_3	5.8167±0.06	1.60±0.07	37±2.64	7.50±0.48	4.58±0.07	43.49±3.01
T_4	5.8433±0.16	1.76±0.05	40±3.60	7.71±0.17	4.95±0.24	39.72±3.65
T_5	5.8567±0.10	1.56±0.05	37.6±3.04	7.80±0.05	4.93±0.13	42.16±3.10
T_6	5.5967±0.52	1.53±0.15	37.3±2.08	7.79±0.17	4.79±0.23	42.95±2.64
T_7	5.8267±0.15	1.60±0.1	35±1	7.67±0.15	4.82±0.17	45.07±0.92

T_0= without antioxidant T_1= BHT (butylated hydroxy toluene)
T_2 (0.015%), T_3 (0.03%), T_4 (0.045%) = Date seed extract
T_5(0.015%), T_6(0.03%), T_7(0.045%) = Fenugreek seed extract

Table 3. Effect of treatments on the antioxidant activity of coconut biscuits

Treatments	Phenolic content	DPPH	Peroxide value
T_0	43.89 ± 4.34^{g}	19.62 ± 2.30^{h}	0.096 ± 0.004^{a}
T_1	85.46 ± 6.44^{b}	49.61 ± 3.87^{b}	0.040 ± 0.002^{e}
T_2	72.08 ± 5.04^{e}	36.39 ± 4.35^{f}	0.072 ± 0.003^{b}
T_3	81.46 ± 5.16^{c}	46.56 ± 4.84^{d}	0.050 ± 0.002^{d}
T_4	92.08 ± 6.02^{a}	52.33 ± 5.10^{a}	0.027 ± 0.001^{f}
T_5	67.55 ± 4.53^{f}	34.21 ± 4.07^{g}	0.061 ± 0.003^{c}
T_6	75.93 ± 4.54^{d}	44.49 ± 4.16^{e}	0.05 ± 0.002^{d}
T_7	85.65 ± 5.03^{b}	48.05 ± 4.23^{c}	0.03 ± 0.001^{f}

Means with similar letters in a column are not significantly different (p>0.01)
T_0= without antioxidant T_1= BHT (butylated hydroxy toluene)
T_2 (0.015%), T_3 (0.03%), T_4 (0.045%) = Date seed extract
T_5(0.015%), T_6(0.03%), T_7(0.045%) = Fenugreek seed extract

Table 4. Effect of storage on the means of antioxidant activity of coconut biscuits

Storage (Days)	Phenolic content	DPPH	Peroxide value
0	80.16±14.50^{a}	46.73±11.16^{a}	0.0525±0.021
15	78.54±15.18^{ab}	43.84±10.9^{b}	0.0529±0.021
30	76.93±14.87^{b}	41.32±10.56^{c}	0.0533±0.021
45	72.94±14.55^{c}	38.81±10.20^{d}	0.0538±0.022
60	68.99±14.15^{d}	36.35±9.53^{e}	0.0554±0.021

Means with similar letters in a column are not significantly different (p>0.01**)**

Table 5. Effect of treatments on the means of physical characteristics of coconut biscuits

Treatments	L*	a*	b*	Texture	Water activity
T_0	88.96±4.10^{a}	2.17±0.08^{a}	12.24±0.47^{a}	2030.8±103.3^{a}	0.236±0.01^{a}
T_1	85.62±3.26^{b}	1.53±0.05^{b}	10.90±0.51^{b}	1977.0±76.4^{ab}	0.208±0.01^{cd}
T_2	85.07±3.23^{bc}	1.29±0.05^{c}	10.13±0.39^{c}	1273.4±50.4^{e}	0.214±0.01^{bc}
T_3	84.64±3.22^{bc}	1.14±0.04^{d}	9.68±0.38^{d}	1581.8±61.9^{c}	0.204±0.01^{de}
T_4	83.95±3.19^{bd}	1.07±0.04^{e}	9.45±0.37^{de}	1893.6±73^{b}	0.198±0.01^{e}
T_5	82.85±3.15^{cd}	0.95±0.04^{f}	9.14±0.35^{ef}	1319.2±58.7^{e}	0.218±0.01^{b}
T_6	81.70±3.11^{de}	0.77±0.04^{g}	9.00±0.36^{fg}	1488.2±83.3^{d}	0.208±0.01^{cd}
T_7	79.83±3.04^{e}	0.64±0.03^{h}	8.80±0.34^{g}	1891.8±87.9^{b}	0.202±0.01^{de}

Means with similar letters in a column are not significantly different (p>0.01)
T_0= without antioxidant T_1= BHT (butylated hydroxy toluene)
T_2 (0.015%), T_3 (0.03%), T_4 (0.045%) = Date seed extract
T_5(0.015%), T_6(0.03%), T_7(0.045%) = Fenugreek seed extract

Table 6. Effect of storage period on the means of the physical characteristics of coconut biscuits

Storage	L*	a*	b*	Texture	Water activity
0	84.31±4.10	1.22±0.45	9.78±1.13	1706±349.1	0.200±0.01^{c}
15	84.21±4.09	1.20±0.46	9.83±1.53	1698.6±348.03	0.205±0.01^{c}
30	84.10±4.09	1.197±0.46	9.91±1.17	1676.4±340.2	0.211±0.01^{b}
45	84.94±4.02	1.190±0.46	10.0±1.19	1669.8±340.08	0.215±0.01^{b}
60	83.81±4.11	1.17±0.47	10.07±1.19	1658.4±340	0.223±0.01^{a}

Means with similar letters in a column are not significantly different (P>0.01)

Table 7. Effect of treatments on the means of sensory characteristics of coconut biscuits

Treatments	Color	Flavor	Taste	Texture	Overall acceptability
T_0	5.81±0.49^{d}	6.00±0.27^{d}	5.35±0.25^{e}	5.76±0.25^{b}	5.26±0.23^{d}
T_1	7.35±0.40^{b}	6.85±0.33^{bc}	6.44±0.28^{c}	6.22±0.27^{a}	6.21±0.26^{ab}
T_2	6.82±0.45^{c}	6.93±0.30^{bc}	6.26±0.28^{cd}	5.91±0.25^{b}	5.72±0.25^{c}
T_3	7.63±0.50^{a}	7.24±0.32^{a}	6.86±0.29^{a}	6.24±0.27^{a}	6.38±0.26^{a}
T_4	5.53±0.31^{e}	5.57±0.29^{e}	4.99±0.27^{f}	5.55±0.24^{c}	4.96±0.22^{e}
T_5	6.66±0.39^{cb}	6.72±0.33^{c}	6.21±0.26^{d}	5.79±0.24^{b}	5.57±0.23^{c}
T_6	7.33±0.36^{b}	7.01±0.32^{b}	6.65±0.29^{b}	6.14±0.26^{a}	6.14±0.26^{b}
T_7	5.34±0.22^{e}	4.76±0.25^{f}	4.96±0.22^{f}	5.23±0.24^{d}	4.84±0.22^{e}

Means with similar letters a column are not significantly different (p>0.01)
T_0= without antioxidant T_1= BHT (butylated hydroxy toluene)
T_2 (0.015%), T_3 (0.03%), T_4 (0.045%) = Date seed extract
T_5(0.015%), T_6(0.03%), T_7(0.045%) = Fenugreek seed extract

Table 8. Effect of storage on means of sensory characteristics of coconut biscuits

Storage days	Color	Flavor	Taste	Texture	Overall acceptability
0	6.77±0.88^{a}	6.63±0.85^{a}	6.16±0.75^{a}	6.03±0.4^{a}	5.80±0.59^{a}
15	6.67±0.90^{ab}	6.50±0.85^{ab}	6.07±0.75^{ab}	5.95±0.4^{ab}	5.72±0.60^{ab}
30	6.55±0.83^{bc}	6.38±0.87^{bc}	5.98±0.75^{bc}	5.85±0.4^{bc}	5.64±0.59^{bc}
45	6.45±0.84^{cd}	6.26±0.85^{cd}	5.86±0.75^{cd}	5.77±0.4^{cd}	5.55±0.60^{cd}
60	6.35±0.83^{d}	6.14±0.85^{d}	5.76±0.75^{d}	5.68±0.38^{d}	5.47±0.59^{d}

Means with similar letters a column are not significantly different (p>0.01)

showed significant differences in a* and b* due to treatments while non-significant decrease and increase during storage period, respectively. The highest L* value was 88.96±4.01 observed in T_o followed by T_1 (85.62±3.26), T_2 (85.07±3.23), T_3 (84.64±3.22), T_4 (83.95±3.19), T_5 (82.85±3.15) and T_6 (81.70±3.11). However, the lowest L* (79.83±3.04) was found in T_7. During storage study, it was observed that there was a gradual decrease in the L* value. The maximum L* (84.31±4.10) was observed at 0 day while the lowest (83.81±4.11) was found at 60th day of storage. Decrease in L* indicated the darkness in color. Similarly, Toma *et al.* (2009) reported the darkening in cookies.

Highest a* 2.17±0.08 was observed in T_o followed by T_1 (1.53±0.05), T_2 (1.29±0.05), T_3 (1.14±0.04), T_4 (1.07±0.04), T_5 (0.95±0.04) and T_6 (0.77±0.04). However, the lowest a* (0.64±0.03) was found in T_7. During storage study, it was observed that there was a gradual decrease in a* value. The maximum a* was observed at 0 day while the lowest was found at 60th day of storage 1.22±0.45 and 1.17±0.47, respectively.

Highest b* 12.24±0.47 was observed in T_o followed by T_1 (10.90±0.51), T_2 (10.13±0.39), T_3 (9.68±0.38), T_4 (9.45±0.37), T_5 (9.14±0.35), T_6 (9.00±0.36). However, the lowest b* (8.80±0.34) was found in T_7. During storage study, it was observed that there was a gradual increase in b* value. The maximum b* (10.07±1.19) was observed at 60th day while the lowest (9.78±1.13) was found at 0 day of storage. Qaisrani *et al*. (2013) reported similar trend for color tonality of cookies.

Texture of coconut biscuits was analyzed with texture analyzer by measuring the force applied on the surface of a biscuit. The results showed that there was a significant effect of treatments on texture of coconut biscuits while storage showed the non-significant effect on texture. Among the treatments, the highest hardness (2030.8±103.3g) was observed in T_o followed by T_1 (1997.0±76.4g), T_4 (1893.6±73g), T_7 (1891.8±87.9g), T_3 (1581.8±61.9g), T_6 (1488.2±83.3g) and T_5 (1319.2±58.7g). However, the lowest hardness (1273.4±50.4g) was found in T_2. During storage study, it was observed that there was a gradual decrease in the texture hardness of coconut biscuits. The maximum hardness was observed at 0 day while minimum was found at 60th day of storage as 1706±349.1 and 1658.4±340g, respectively. The hygroscopic ingredients contribute to decrease the hardness. During storage, the hardness level decreases as the moisture content increases (Mushtaq *et al.*, 2010). Similarly, Wade (1988) stated that texture hardness of biscuits varied considerably according to the composition of biscuits.

The level of water activity in food is a critical control point because it may be used to measure the bacterial growth potential and spoilage occurs during storage. The results showed that there was a significant effect of treatments and storage periods on water activity of coconut biscuits. Among the treatments, the highest water activity (0.236±0.01) was observed in T_o followed by T_1 (0.208±0.01), T_2 (0.214±0.01), T_6 (0.208±0.01), T_5 (0.218±0.01), T_3 (0.204±0.01), T_7 (0.202±0.01) and T_4 (0.198±0.01). During storage studies, it was observed that there was a gradual increase in water activity of biscuits. The minimum water activity was observed at 0 day while maximum was found at 60th day of storage 0.200±0.01 and 0.223±0.01, respectively. Mushtaq *et al.* (2010) also reported the similar values of water activity for cookies and concluded that water activity increased during storage. Eisa (2006) reported that water activity plays an important role in shelf life of bakery products and facilitates microbial growth at higher activity (>0.8).

Sensory evaluation

Coconut biscuits were evaluated on the basis of color, taste, flavor, texture and overall acceptability by following the sensory evaluation performa. The results on these characteristics are presented in the Tables 7 and 8. The results showed that treatments and storage periods have significant effects on the color of coconut biscuits. It is evidenced from the table that score for the color was found the highest for T_3 (7.63±0.50) and the lowest was observed for T_7 (5.34±0.22) among treatments. Scores for other treatments were recorded as 7.35±0.40 for T_1, 7.33±0.36 for T_6, 6.82±0.45 for T_2, 6.66±0.39 for T_5, 5.81±0.49 for T_o and 5.53±0.31 for T_4. Color scores showed a decreasing trend during storage. The maximum score (6.77±0.88) was obtained at 0 day, while the minimum score (6.35±0.83) was obtained at 60th day. The results are found agree with the findings of Manley (2002) who reported that color of the biscuits became fade during storage which might be happened due to the light and other physicochemical changes occurred during storage.

Treatments and storage periods have a significant effect on the flavor. Score for the flavor was found the highest for the T_3 (7.24±0.32) and the lowest was shown by T_7 (4.76±0.25). Scores for other treatments were recorded as 7.01±0.32 for T_6, 6.93±0.30 for T_2, 6.85±0.33 for T_1, 6.72±0.33 for T_5, 6.00±0.27 for T_o and 5.57±0.29 for T_4. Flavor scores showed a decreasing trend during storage. The maximum score (6.63±0.85) was obtained at 0 day, while the minimum score (6.14±0.85) was obtained at 60th day. The results are found in accordance with the findings of Sharif *et al*. (2009) who also reported the decreasing trend of flavor during storage. This may be due to oxidation of fat that occurred due to moisture absorption.

Effect of treatments and storage was also found significant on the taste of coconut biscuits. Score for the taste among treatments was found the highest for the T_3 (6.86±0.29) and the lowest was shown by T_7 (4.96±0.22). Scores for other treatments were recorded as 6.65±0.29 for T_6, 6.44±0.28 for T_1, 6.26±0.28 for T_2, 6.21±0.26 for T_5, 5.35±0.25 for T_o and 4.99±0.27 for T_4. Taste scores showed a decreasing trend during storage. The maximum score (6.16±0.75) was obtained at 0 day, while the minimum score (5.76±0.75) was obtained at 60th day. The results are found in accordance with the findings of Nassar *et al.* (2008) and Sharif *et al.* (2009). Decrease in score of taste may be due to the hydrolytic and oxidative changes occurred during storage.

Results showed that treatments and storage have the significant effects on the crispiness/texture of coconut biscuits. Score for the crispiness among treatments was the highest for the T_3 (6.24±0.27) and the lowest were shown by T_7 (5.23±0.24). Scores for other treatments were recorded as 6.22±0.27 for T_1,

6.14±0.26 for T_6, 5.91±0.25 for T_2, 5.79±0.24 for T_5, 5.76±0.25 for T_o and 5.55±0.24 for T_4. Scores of crispiness showed a decreasing trend during storage. The maximum score (6.03±0.4) was obtained at 0 day, while the minimum score (5.68±0.38) was obtained at 60th day. Crispiness might be reduced due to increase in content of moisture. Score for the overall acceptability among treatments was the highest for the T_3 (6.38±0.26) and the lowest was shown by T_7 (4.84±0.22). Scores for other treatments were recorded as 6.21±0.26 for T_1, 6.14±0.26 for T_6, 5.72±0.25 for T_2, 5.57±0.23 for T_5, 5.26±0.23 for T_o and 4.96±0.22 for T_4. Scores of overall acceptability showed a decreasing trend during storage. The maximum score 5.80±0.59 was obtained at 0 day. While the minimum score (5.47±0.59) was obtained at 60th day. The results are found in accordance with the findings of the Nassar *et al.* (2008), Sharif *et al.* (2009) and Aruoma (1998).

CONCLUSIONS

It is evident from the present research work that coconut biscuits containing natural antioxidants showed better and comparable results with the artificial antioxidants. Coconut biscuits prepared by adding date seed and fenugreek seed extracts at a level of 0.03% could be store upto 60 days without affecting their sensory properties. There was not a noticeable odor of rancidity found in coconut biscuits during storage at ambient temperature.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. AACC. 2003. All dietary fiber is fundamentally functional; an AACC report. Cereal Food World., 48: 128-131.
2. Acharya, S., A. Srichamreon, S. Basu, B. Ooraikul and T. Basu. 2006. Improvement in the nutraceutical properties of fenugreek (*Trigonella foenum-graecum L.*) Songklanakarin. J. Sci., 28:1-9.
3. Ajila, C. M., R. L. Jaganmohan and U. J. S. P. Rao. 2010. Characterization of bioactive compounds from raw and ripe *Mangifera indica* L. peel extracts. Food Chem. 1141-1152.
4. Al-Farsi, M., C. Alasalvar, M. Al-Abid, K. Al-Shoaily, M. Al-Amry and F. Al-Rawahy. 2007. Compositional and functional characteristics of dates, syrups, and their by-products. Food Chem., 104:943-947.
5. Al-Hooti, S. N., J. S. Sidhu, J. M. Al-Saqer and A. Al-Othman. 2002. Chemical composition and quality of date syrup as affected by pectinase/cellulase enzyme treatment. Food Chem., 79: 215-220.
6. AOAC. 2006. Official Methods of Analysis of Association of Official Analytical Chemists. 18th Ed. AOAC press, Arlington, VA, USA.
7. Aruna, M.C. 2000. Development of vitamin fortified soy biscuits and its effect on the nutritional status of selected school children. MSc Thesis, Deptt. HSC and RI. Univ. Agric., India.
8. Aruoma, O. I. 1998. Free radicals, oxidative stress and antioxidants in human health and disease. J. Am. Oil Chem. Soc., 75:199-212.
9. Dhankar, P. 2013. A study on development of coconut based gluten free cookies. Int J. Engg. Sci. Invent., 2:10-19.
10. Eisa, H. A. 2006. The effect of using gluten free flours on the palatability, texture and water activity of white chocolate chip Macadamia Nut Cookies. Individual project written report, Food and nutrition. 453.
11. Hajimehdipoor H., S.E. Sadat-Ebrahimi, Y. Amanzadeh, M. Izaddost and E. Givi. 2010. Identification and quantitative determination of 4-hydroxyisoleucine on *Trigonella foenum-graecum* from iran. J. Med. Plants., 9:29-34.
12. Iqbal, S., M. I. Bhanger, and A. Farooq. 2005. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. Food Chem., 93:265-272.
13. Joseph, J. A., B. Shukitt-Hale and G. Casadesus. 2005. Reversing the deleterious effects of aging on neuronal communication and behavior: Beneficial properties of fruit polyphenolic compounds. Am. J. Clin. Nutr., 81:313-316.
14. Juhaimi, F. A., K. Ghafoor and M. M. Özcan. 2012. Physical and chemical properties, antioxidant activity, total phenol and mineral profile of seeds of seven different date fruit *(Phoenix dactylifera L.)* varieties. Int. J. Food Sci. Nutr., 63:84-89.
15. Kaur, J., H. Singh and M. U. Khan. 2011. Multifarious therapeutic potential of fenugreek: A comprehensive review. Int. J. Res. Pharma. Biomed. Sci., 2:863-871.
16. Kaviarasan, S., K. Vijayalakshmi and C. V. Anuradha. 2004. A polyphenol rich extract of fenugreek seeds protect erythrocytes from

oxidative damage. Plant Foods Hum. Nutr., 59:143-147.

17. Kilcast, D., and P. Subramaniam. 2000. The stability and shelf-life of food. 2nd Ed. Woodhead Pub. Ltd. North and South America. pp. 302.
18. Kumar, K. I. M. and B. P. Maliakel. 2008. Fenugreek dietary fiber novel class of functional food ingredient. Agro. Food Ind. Hi-Tech., 19:18-21.
19. Lawless, H.T. and H. Heymann. 2010. Sensory Evaluation of Food: Principles and practices. 2nd Ed. New York Springer. New York, NY, USA. pp. 85-103.
20. Magda, R. A., A. M. Awad and K. A. Selim. 2008. Evaluation of mandarin and navel orange peels as natural sources of antioxidant in biscuits. In. Alex. J. Food Sci. Technol. 5: 75-82.
21. Manisha, G., C. Soumya and D. Indrani. 2012. Studies on interaction between stevioside, liquid sorbitol, hydrocolloids and emulsifiers for replacement of sugar in Muffin. Food Hydrocoll., 29:363-373.
22. Manley, D., 2002. Technology of biscuits, crackers and cookies, 3rd Ed. Woodhead Pub. Ltd. Cambridge, UK. pp. 191.
23. Mushtaq, Z., S. Rehman, T. Zahoor and A. Jamil. 2010. Impact of xylitol replacement on physicochemical, sensory and microbial quality of cookies. Pak. J. Nutr., 9:605-610.
24. Naidu, M. M., B. N. Shyamala, J. P. Naik, G. Sulochnamma and P. Srinivas. 2011. Chemical composition and antioxidant activity of the husk and endosperm of fenugreek seeds. Food Sci. Technol., 44:451-456.
25. Nanditha, B. and P. Prabhasankar. 2008. Antioxidants in bakery products: A Review. Crit. Rev. Food Sci. Nutr., 49:1-27.
26. Nassar, A. G., A. A. AbdEl-Hamied and E. A. El-Naggar. 2008. Effect of citrus by-products flour incorporation on chemical, rheological and organoleptic characteristics of biscuits. World J. Agric. Sci. 4:612-616.
27. Platat, C., H. M. Habib, F. D. AL Maqbali, N. N. Jaber, W. H. Ibrahim. 2014. Identification of date seeds varieties patterns to optimize nutritional benefits of date seeds. J. Nutr. Food Sci. 8: 99-111.
28. Priya, S. R. and L. Ramaswamy. 2016. Physical, chemical and microbial properties of cookies developed using coconut products. Int. J. Rec. Sci. Res., 7:9670-9674.
29. Qaisrani, T. B., M. S. Butt, F. M. Anjum, and M. A. Sheikh. 2013. Color tonality and sensory response of Psyllium husk based cookies. Pak. J. Nutr., 12:55-61.
30. Rocha, A. M. C. N. and A.M.M.B. Morais. 2003. Shelf life of minimally processed apple determined by color change. Food Control., 14:13-20.
31. Sadasivam, S. and A. Manickam. 2008. Biochemical Methods. 2nd Ed. New age International Publishers, New Delhi, India. pp. 193-200.
32. Seevaratnam, V. P. B., M. R. Premalatha, S. P. Sundaram and T. Arumugam. 2012. Studies on the preparation of biscuits incorporated with potato flour. World J. Dairy Food Sci., 7:79-84.
33. Sharif, M. K., M. S. Butt, F. M. Anjum and H. Nawaz. 2009. Preparation of fiber and mineral enriched defatted rice bran supplemented cookies. Pak. J. Nutr., 8:571-577.
34. Sidhu, J.S., N. Suad and M. Jameela. 1999. Effect of adding wheat bran on the chemical composition of muffins. J. Food Chem., 67:365-371.
35. Tadhani, M. and R. Subhash. 2007. Preliminary studies on *Stevia rebaudiana Leaves*: Proximate composition, mineral analysis and phytochemical screening. J. Med. Sci. 6:321-326.
36. Toma, A., M. B. Omary, L. F. Marquart, E. A. Arndt, K. A. Rosentrater, B. Burns-Whitmore, L. Kessler, K. Hwan, A. Sandoval and A. Sung. 2009. Children's acceptance, nutritional and instrumental evaluations of whole grain and soluble fiber. J. Food Sci. 74: 139-146.
37. Tyagi, S. K., M. R. Manikantan, S. O. Harinder and G. Kaur. 2007. Effect of mustard flour incorporation on nutritional, textural and organoleptic characteristics of biscuits. J. Food Eng., 80:1043-1050.
38. Verardo, V., Y. Riciputi, M. Cristina, M. Vallicelli, L. Falasca, E. Marconi and M. F. Caboni. 2011. Dietary fiber and flavan-3-ols in shortbread biscuits enriched with barley flours co-products. J. Food Sci. Nutri., 62:262-269.
39. Wade, P., 1988. Biscuits, cookies and crackers. Vol. 1. Elsevier Applied Sciences. London, UK. pp.7-51.

PAK. J. FOOD SCI., 27(1), 2017: 26-32
ISSN: 2226-5899

Study of inhibitory spectrum of metabolic extract from *Saccharomyces boulardii* yeast against some food related bacteria

[1]Alaa Kareem Naimah, [1]Alaa Jabbar Abd Al–Manhel and [1]Manar Jabbar Al-Shawi

[1]Department of Food Science, College of Agriculture, University of Basrah, Basrah City, Iraq

Corresponding author: alaa.niamah@uobasrah.edu.iq

ABSTRACT

The current study was carried out for testing the inhibitory spectrum of the metabolic extract of *Saccharomyces boulardii* yeast against (26) bacterial isolates. The metabolic extract of *Saccharomyces boulardii* yeast was obtained after growth in YEPG medium and centrifuged. The broad inhibitory spectrum of yeast extract against Gram-positive bacteria and Gram-negative bacteria assay by liquid medium method and solid medium method. The highest inhibition of *Enterobacter* spp. and *B. cereus* in the solid medium were (37) mm and (35) mm respectively when adding the inhibitory substances at (100 µL) concentration while the inhibition ratio percentage in the liquid medium was (74.2%) and (72.1%) respectively for both bacteria. Less inhibition diameter for bacteria was *E. coli* 6 which reaches (18) mm. after adding (100µl) concentrations, while the percentage of inhibition in the liquid medium was (26.7%).

Keywords: *Saccharomyces boulardii*, inhibitory spectrum, food-spoiling bacteria

INTRODUCTION

The World Health Organization (WHO)and The Food and Agriculture Organization (FAO) have defined a general definition stating that the cells that are able to cross the upper regions of the digestive system and have intestinal growth when given in high numbers, for the purpose of health effects on the host who taken them (FAO/WHO, 2002). Yeast is counted as one of the important microbial organisms that producing (peptides), especially those of the *Saccharomyces* species. It capable of producing vital biological substances that can be involved in many different kinds of activities, especially in the possibility of being used as (probiotics) such as *Saccharomyces boulardii* yeast, which can produce different inhibitory substances for not only beneficial for the health of the gastrointestinal tract but can be maintained for longer period in the gastrointestinal tract in comparison with the *Saccharomyces cerevisiae* (Qamar *et al*.,2001; Liu *et al*., 2016).

The French scientist Henri Boulard discovered *Saccharomyces boulardii* in 1923 which isolated from lychee fruit *Litchi chinensis* and Mangosteen fruit *Garcinia mangostana* (Zbar *et al*., 2013). *S. boulardii* yeast accounted as one of the most important types of living therapeutic diseases that have been used to improve the intestinal system by preventing inflammatory bowel infections such as *Salmonella typhimurium, Clostridium difficile*, Escherichia *coli*, Candida *albicans* in the epithelial cells as well as treating a number of diarrhea cases acute in children and gastrointestinal disorders caused by antibiotics (Tiago *et al*., 2012; Tomičić *et al*., 2016a). The *S. boulardii* yeast was characterized by its important ability to grow at a temperature of 37°C and with acidity of the stomach, did not have any effect against the natural germination of the gastrointestinal tract (GIT), and is not affected by the presence of antibiotics. Therefore, it has been widely used as living therapeutic animals to support the health of the digestive system (Tomičić *et al*., 2016b).

Alzubaidy and Khidhr (2014) pointed out the activity of *Saccharomyces boulardii* yeast, which inhibits many pathogenic bacteria. 50 isolates of *Saccharomyces cerevisiae* var. *boulardii* was obtained fresh and dried fruits and their inhibitory activity test against *Salmonella enteric* subsp. *enterica* by well diffusion method. The most yeast isolates showed inhibitory effectiveness against the bacteria test and the highest diameter of inhibition zones was 16 mm. The aim of the study, identification of the inhibitory spectrum of *Saccharomyces boulardii's* metabolic extract for different types of bacteria that are related to food spoilage.

MATERIALS AND METHODS

The yeast source

The *Saccharomyces boulardii* ATCC MYA® 796 ™ standard that is processed and supplied from the Swanson probiotic laboratories of Australia.

Sources of bacterial isolates

Twenty-six bacterial isolates were obtained from College of Nursing, College of Dentistry, College of Science and College of Agriculture were used to study the inhibitory action of the inhibitory metabolic extract produced from *S. boulardii* yeast.

Activation of yeast

The yeast was activated by planting 0.1 g of dried yeast in sterile distilled water for 30 min at 30°C and then placed on the vortex mixer. 1mL of yeast was transferred to a conical flask content 100 mL of YEPG medium that supplied of Himedia Company/ Indian. It was prepared by solving 50 g of the medium in liter of distilled water and pH at 5 ± 0.2 and sterilized at 1.5 bar for 20 minutes. The conical flask were incubated at 30°C for 24 hours. The number of viability cells of yeast counted by pour plate method, which was 47 × 10^8 colony formation unit/mL (CFU/ mL).

Activation of bacteria

Bacterial isolates were activated by planting 0.1 mL of bacteria in 5 mL of nutrient broth medium obtained from Salucea Dutch Company that was prepared by solving 13g of medium in one liter distilled water with pH 7.2 ± 0.2, incubated at 37 °C for 18–24 hr. The viable bacteria cells were counted using pour plate method that ranged from 10^6-10^8 CFU / mL (Niamah and Alali, 2016).

The metabolic extract of yeast

Active yeast was grown by 0.5 ml / 100 mL from the media of YEPG. The conical flask was incubated at 30°C for 24 hours. After the incubation period, then carried out the centrifugation at 3000 rpm / min for 15 min then the extract of yeast was filtered (0.45µL of millipore filter). After taking the supernatant (cell free) and neglected sludge (Ali *et al*., 2012).

Inhibitory activity of yeast metabolic extract

Inhabitation in liquid medium method

Media was prepared and incubated as per guidelines of Niamah, 2010. The supernatant of the activated yeast was added to the activated bacteria test to 3mL of Nutrient broth that was containing 10^6-10^8 CFU / mL. The mixed of ratio (1:1) and incubated at 37°C for 24 hours. The control sample of bacteria test was not adding the metabolic extract of yeast. The growth density was measured by the optical spectrometer at 600 nm. The inhibitory activity was estimated and calculated as the percentage of inhibition as in the equation below:

% inhibition = (Sample without metabolic extract - Bacteria test) / Sample without metabolic extract × 100)

Inhabitation in solid medium method

The well diffusion agar method (Niamah, 2014; Al-manhel and Niamah, 2015) to reveal of the inhibitory action of yeast filtration by spreading 0.1mL of containing 10^6-10^8 CFU / mL of the bacterial isolates test on the Muller-Hinton Agar medium (Himedia/ India) by a sterilized glass rod (L–shape) that made three-wells in each dish and a diameter of (6 mm) sterilized cork borer. Added three concentrates of (50, 75 and 100) µL of the yeast metabolic extract and placed in the refrigerator for two hours. Then, after that incubating, the dishes at 37°C for 24 hours and then the area of inhibition or the zones was measured.

Statistical Analysis

Statistical analysis carried out according to method described by Dean and Voss (1999) and data expressed as mean ± SD of three replicates.

RESULTS AND DISCUSSION

Figure (1) shows the inhibitory effect of the metabolic extract from *S. boulardii* yeast against the food-spoiling bacteria. The inhibitory activity was tested on liquid medium and solid medium by the well diffusion agar method. The results showed wide degree of inhibition of this yeast against different types of bacteria that were isolated from the different sources. The highest inhibition percentage against the tested bacteria Enterobacteria spp. and *Bacillus cereus*. The inhibition percentage in the liquid medium were (74.2% and 72.1%) respectively. The lowest inhibition percentage against the *E. coli* 6 and *Staphylococcus aureus*. They were 26.7% and 32.7%, respectively.

Results also showed the used well diffusion agar method in the solid medium that the increased concentration of yeast extract led to increased diameters of the types of bacteria that are pathological or related to food. It was observed that when adding 100µL of inhibitory metabolic extract in the well zones that the rate of the inhibition diameter of the *Enterobacteria* spp. and *Bacillus cereus* were (37 and 35) mm respectively. While the diameters of 18 mm and 25 mm were inhibition of *E. coli* 6 and *Staphylococcus aureus*1 bacteria as shown in Fig. 2 and the Table (2). The difference in the effect of inhibition on bacterial cells is due to the different receptors that are found in the walls of bacterial cells, as well as the different of the mechanism of action of inhibitory substances and their effect on the cell wall through making zones or openings in the cells walls (Niamah, 2010). The resulting of the loss of the ions and the destruction of the plasma membrane, as well as its ability to production of bores serene which has been found proving its effect action on the analysis of the proteins that were found in the external walls of the cells, as well as upon some of the metabolic substances in the yeast extract upon the active intracellular transport systems (Zbinden, 1999). The results are agreed with Ali *et al.* (2012) in yeast extract of S. *boulardii* was contained the antimicrobial agents

Table 1. Bacterial isolates used in estimation of inhibitory spectrum of inhibitory substances from *Saccharomyces boulardii* yeast in liquid and solid media

No.	Bacterial isolation	Source	Educational Institution
1	*Bacillus cereus*	Soil	College of Agriculture /University of Basrah
2	*Bacillus licheniformis*	Soil	College of Agriculture / University of Basra
3	*Citrobacter spp.*	Fish	College of Agriculture University of Basrah
4	*Escherichia coli*[1]	Milk	College of Veterinary Medicine /University of Basrah
5	*Escherichia coli*[2]	Milk	College of Veterinary Medicine / University of Basrah
6	*Escherichia coli*[3]	Milk	College of Veterinary Medicine/University of Basrah
7	*Escherichia coli*[4]	Fish	College of Agriculture /University of Basrah
8	*Escherichia coli*[5]	Water	College of Center Marine Sciences /University of Basrah
9	*Escherichia coli*[6]	Teeth	College of Dentistry /Basrah
10	*E. coli* O_{157}: H_7	Meat	College of Agriculture / University of Basrah
11	*Enterobacter spp.*	Fish	College of Agriculture / University of Basrah
12	*Klebsiella pneumoniae*[1]	Burns	College of Nursing / University of Basrah
13	*Klebsiella pneumoniae*[2]	Fish	College of Agriculture /University of Basrah
14	*Kocuria kristinae*	Water	College of Center Marine Sciences /University of Basrah
15	*Micrococcus* spp.	Fish	College of Agriculture /University of Basrah
16	*Pseudomonas aeruginosa*1	Milk	College of Education /University of Basrah
17	*Pseudomonas aeruginosa*[2]	Burns	College of Nursing /Basrah
18	*Pseudomonas aeruginosa*[3]	Water	College of Center Marine Sciences /University of Basrah
19	*Pseudomonas aeruginosa*[4]	Fish	College of Agriculture / University of Basrah
20	*Pseudomonas aeruginosa*[5]	Soil	College of Sciences/University of Basrah
21	*Staphylococcus aureus*[1]	tonsillitis	College of Nursing / University of Basrah
22	*Staphylococcus aureus*[2]	Water	College of Center Marine Sciences /University of Basrah
23	*Staphylococcus aureus*[3]	Fish	College of Agriculture /University of Basrah
24	*Staphylococcus aureus*[4]	Soil	College of Sciences/University of Basrah
25	*Staphylococcus aureus*[5]	Teeth	College of Dentistry / University of Basrah
26	*Vibrio cholerae*	Fish	College of Agriculture / University of Basrah

Table 2. Inhibitory spectrum of Saccharomyces *boulardii* yeast extract against bacterial species in solid media

No.	Bacterial Isolations	Effectiveness of inhibitory metabolic extract of *Saccharomyces boulardii* yeast in solid media		
		Diameter zones of inhibition (mm)*		
		100 µL	75 µL	50 µL
1	*Bacillus cereus*	35±0.03	29±0.03	27±0.04
2	*Bacillus licheniformis*	24±0.06	22±0.05	17±0.01
3	*Citrobacter spp.*	23±0.02	21±0.02	17±0.09
4	*Escherichia coli*[1]	29±0.03	26±0.01	24±0.08
5	*Escherichia coli*[2]	27±0.02	24±0.08	22±0.02
6	*Escherichia coli*[3]	23±0.07	20±0.00	17±0.02
7	*Escherichia coli*[4]	23±0.09	20±0.02	17±0.06
8	*Escherichia coli*[5]	34±0.01	29±0.07	25±0.04
9	*Escherichia coli*[6]	18±0.05	15±0.01	12±0.03
10	*E. coli* O_{157}: H_7	22±0.02	18±0.08	15±0.09
11	*Enterobacter spp.*	37 ±0.02	34±0.03	30±0.08
12	*Klebsiella pneumoniae*[1]	28±0.05	25±0.05	23±0.05
13	*Klebsiella pneumoniae*[2]	27±0.07	25±0.07	22±0.05
14	*Kocuria kristinae*	28±0.03	25±0.04	22±0.07
15	*Micrococcus* spp.	22 ±0.04	20 ±0.01	18 ±0.04
16	*Pseudomonas aeruginosa*1	24±0.04	22±0.09	19±0.00
17	*Pseudomonas aeruginosa*[2]	24±0.01	22±0.04	18±0.05
18	*Pseudomonas aeruginosa*[3]	30±0.09	27±0.02	25±0.04
19	*Pseudomonas aeruginosa*[4]	28±0.10	25±0.09	22±0.06
20	*Pseudomonas aeruginosa*[5]	21±0.08	19±0.06	16±0.02
21	*Staphylococcus aureus*[1]	25±0.03	22±0.02	20±0.09
22	*Staphylococcus aureus*[2]	27±0.02	24±0.05	21±0.04
23	*Staphylococcus aureus*[3]	23±0.01	19±0.06	15±0.07
24	*Staphylococcus aureus*[4]	24±0.03	20±0.03	18±0.05
25	*Staphylococcus aureus*[5]	24±0.06	21±0.02	19±0.09
26	*Vibrio cholerae*	21±0.02	19±0.05	16±0.02

Data are Means ± SD, *No. of repeaters = 3

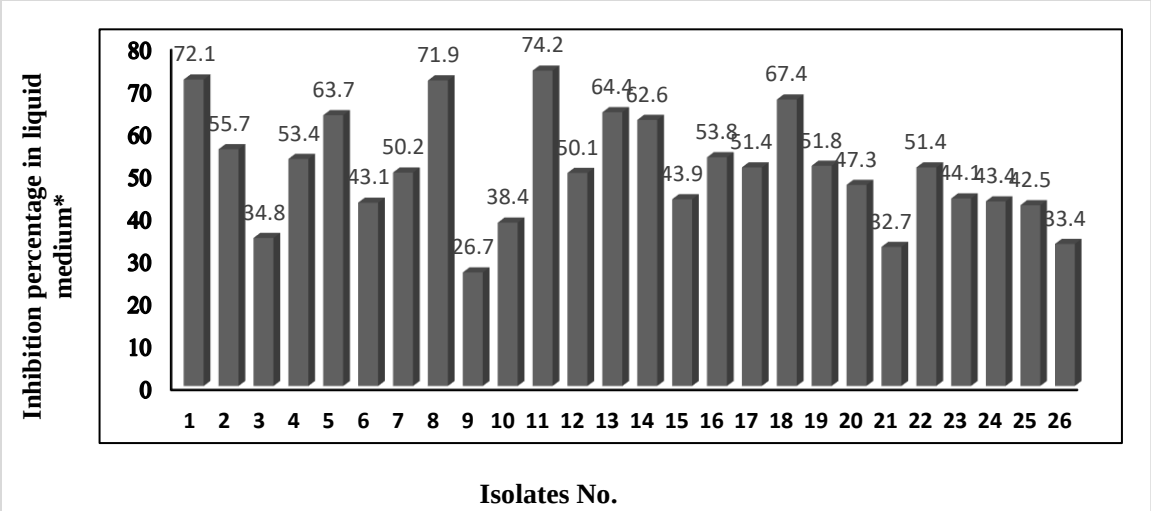

Fig. 1. The inhibitory spectrum of *S. boulardii* yeast extract against the bacterial species in the liquid medium (*No. of repeaters = 3)

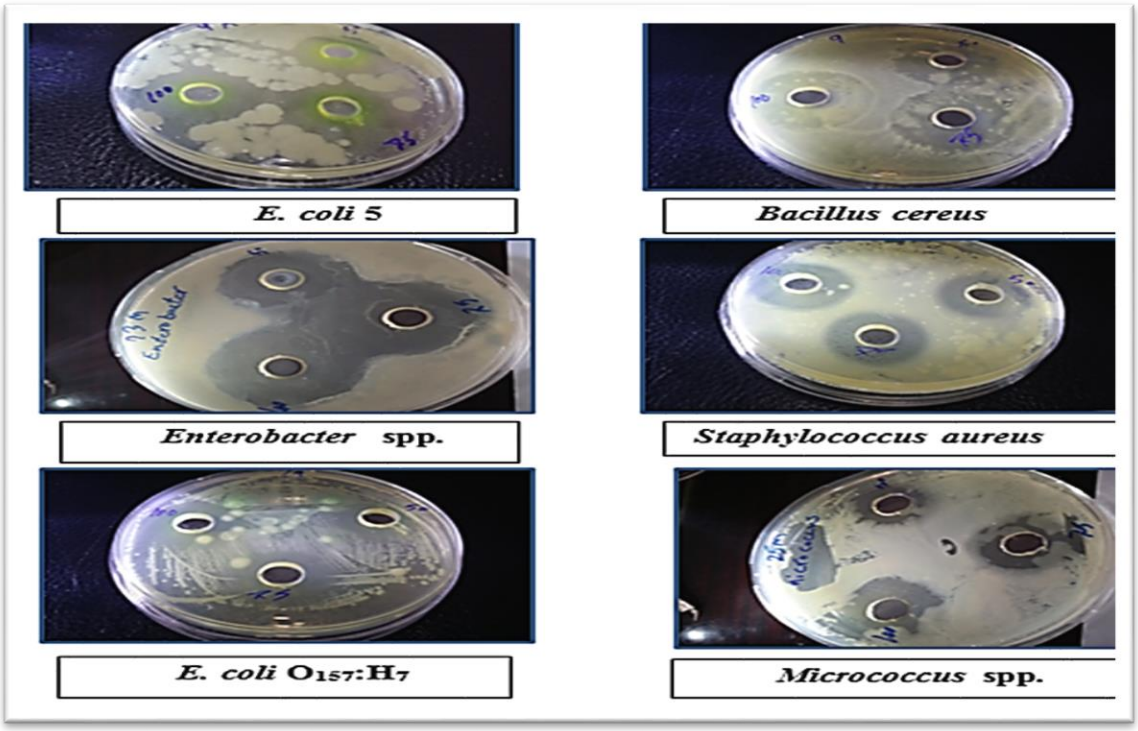

Fig. 2. Diameters of the inhibitors of *S. boulardii* yeast extract towards bacterial isolates

which show the inhibitory activity of the extract against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus mycoides*, *Escherichia coli* and *Candida albicans*. The diameters of the inhibition zone were (19, 17, 18, 14 and 15 mm), respectively.

The study of the effectiveness of seven isolates of yeast *Saccharomyces cerevisiae* var. *boulardii* against various diarrheal pathogens, this yeast has inhibitory ability against a variety of pathogenic bacteria and the number of bacteria that causes diseases. It has decreased significantly in the mixed cultures that composed bacteria of *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. It shows the decrease rate in number was between 55.9 % in comparison with the pure cultures that are containing bacteria of *Listeria monocytogenes* only, which reached 22.5% (Rajkowska and Kunicka-

Styczyńska, 2012). Additionally, Sharma and Upadhya (2015) found that the metabolic yeast extract of *S. boulardii* had an inhibitory effect against *Pseudomonas*, *E. coli* and Staphylococcus when using the inhibition method of the discs which reached the inhibitory diameters (13, 14, 19) mm, respectively.

Additionally, Syal and Vohra (2014) isolated yeast with antimicrobial activity against *E. coli*, *Salmonella* sp., *Staphylococcus aureus*, *Vibrio cholerae* and *Pseudomonas* sp. Further studies on antimicrobial activity of the yeast isolate against other species of pathogenic bacteria and fungi are needed. When studying (Alwan *et al.*, 2014) show the inhibitory effect of two types of probiotic organisms, *Lactobacillus acidophilus* and *Saccharomyces boulardii* against the bacteria of *Staphylococcus aureus* in the solid medium by the well diffusion agar method. The inhibitory diameter of *S. boulardii* was 33 mm while the inhibition diameter of *Lactobacillus acidophilus* was 15 mm.

CONCLUSION

Saccharomyces boulardii is one of the most studied microorganisms. It has long been used in various biotechnology applications because of its ability to ferment better. Besides industrial applications, it has also been reported probiotic health and potential benefits of yeast in recent. The yeast has inhibition activity against more microorganisms. The metabolic extract of *Saccharomyces boulardii* have a large inhibitory effect on many of bacteria of food spoilage and pathogenic bacteria. The inhibitory effect of the metabolic extract is increased by increasing the added concentrations while inhibition activity in the liquid medium were consistent with the results in the solid medium. The metabolic extract of *S. boulardii* can be used as a natural alternative to chemicals preservative and adding some kinds of food.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

I would like to express my special thanks of gratitude to Biotechnology Lab. College of Agriculture, University of Basrah, The Ministry of Higher Education and Scientific Research, Iraq.

REFERENCE

1. AL Zubaidy, Z.M. and K.O. Khidhr. 2014. Isolation and Identification of *Saccharomyces cerevisiae* var. *boulardii* and its Uses as a Probiotic (*in vitro*). Raf. J. Sci. 25(10): 1-11.
2. Ali, M., O. Abdel-Fatah, J.C. Janson and A. Elshafei. 2012. Antimicrobial potential of *Saccharomyces boulardii* extracts and fractions. J. App. Sci. Res. 8(8): 4537-4543.
3. Al-Manhel, A.J. and A. K. Niamah. 2015. Effect of aqueous and alcoholic plant extracts on inhibition of some types of microbes and causing spoilage of food. Pak. J. Food Sci. 25(3): 104-109.
4. Alwan, A.H., A. Haider Alsakini, and F.N. Abdulrassak. 2014. The protective effect of probiotics (*Lactobacillus acidophilus* and *Saccharomyces boulardii*) against infections caused by *Staphylococcus aureus in vitro* and *in vivo*. Int. J. Curr. Microbiol. App. Sci. 3(7): 886-890.
5. Dean, A. and D. Voss. 1999. Design and analysis of experiments, Springer – verlag. New York, Inc. U.S.A. 740p.
6. FAO/WHO, 2002. Guidelines for the evaluation of probiotics in food. Food and agriculture organization of The United Nations/World Health Organization, London, Ontario Canada. pp.11.
7. Liu, J. J., I. I. Kong, G. C. Zhang, L. N. Jayakody, H. Kim, P. F. Xia, S. Kwak, B. H. Sung, J. H. Sohn, and H. E.Walukiewicz. 2016. Metabolic engineering of probiotic *Saccharomyces boulardii*. Appl. Environ. Microbiol. 82(8): 2280-2287.
8. Niamah, A. K. 2010. Production of pediocin like bacteriocin from a local isolate of *Pediococcus acidilactici* and using it as foods preservative. Ph.D. thesis, College of Agriculture, University of Basrah, 177p.
9. Niamah, A. K. 2014. Determination, identification of bioactive compounds extracts from yellow banana peels and used in vitro as antimicrobial. International Journal of Phytomedicine. 6(4): 625-632.
10. Niamah, A. K. and H.A. Alali. 2016. Antibacterial and antioxidant activities of essential oils extracted from Iraqi coriander *(Coriandrum sativum* L.) seeds Int. J. Sci. Eng. Res. 7(2): 1511-1515.
11. Qamar, A., S. Aboudola, M. Warny, P. Michetti, C. Pothoulakis, J.T. Lamont, and C.P. Kelly. 2001. *Saccharomyces boulardii* stimulates intestinal immunoglobulin a immune response to clostridium difficiletoxin a in mice. Infect. Immun. 69(4): 2762-2765.
12. Rajkowska, K. and A. Kunicka-Styczyńska. 2012. Probiotic activity of *Saccharomyces cerevisiae* var. *boulardii* against human pathogens. Food Technol. Biotech. 50 (2): 230-236.
13. Sharma, J. and S. Upadhya. 2015. Determination of antimicrobial potential of *Saccharomyces boulardii* and *Bacillus clausii* against some community acquired pathogens *in vitro* study. Int. J. Pharm. Sci. Res. 6(7): 1023-1026.
14. Syal, P., A.Vohra. 2014. Probiotic attributes of a yeast-like fungus, *Geotrichum klebahnii*. Afr. J. Microbiol. Res. 8(20):2037–43.
15. Tiago, F. D. C. P., F. D. S.Martins, E. Souza, P. F. P. Pimenta, H. R. C. Araújo, I. D. M. Castro, R. L. Brandão, and J. R. Nicoli. 2012. Adhesion to the yeast cell surface as a mechanism for trapping pathogenic bacteria by *Saccharomyces* probiotics. J. Med. Microbiol. 61(9): 1194-1207.

16. Tomičić, R. M., I. S. Čabarkapa, Đ. M. Vukmirović, J. D. Lević, and Z. M. Tomičić. 2016a. Influence of growth conditions on biofilm formation of *Listeria monocytogenes*. Food Feed Res. 43 (1): 19-24.
17. Tomičić, Z. M., R. R. Čolović, I. S. Čabarkapa, Đ. M. Vukmirović, O. M. Đuragić, and R. M. Tomičić. 2016b. Beneficial properties of probiotic yeast *Saccharomyces boulardii*. Food Feed Res. 43 (2):103-110.
18. Zbar, N.S., L.F. Nashi and S. M. Saleh. 2013. *Saccharomyces boulardii* as effective probiotic against *Shigella flexneri* in mice. Int. J. Mater. Methods Technol. 1:17–21.
19. Zbinden, R. 1999. Inhibition of *Saccharomyces boulardii* (nom. inval.) on cell invasion of *Salmonella typhimurium* and *Yersinia enterocolitica*. Microb. Ecol. Health Dis.11 (3): 158-162.

PAK. J. FOOD SCI., 27(1), 2017: 39-45
ISSN: 2226-5899

Enrichment of wheat flour bread to enhance physicochemical and sensory attributes using broccoli powder

Baqar Rasul Anwar, Allah Rakha, Muhammad Arsalan Mahmood, Iffat Batool, Muhammad Sohail and Sammar Rashid

National Institute of Food Science and Technology, University of Agriculture, Faisalabad. 38040-Pakistan

Corresponding Author: arsalan7590@yahoo.com

ABSTRACT

Enrichment of wheat flour with dehydrated broccoli powder has positive effects on the nutritional value of bread. In present study, broccoli powder was prepared by dehydrating the broccoli and then added in wheat flour in variable proportions (1%, 3%, 5% and 7%) to check its impact on physico-chemical, rheological and sensory attributes of enriched bread. Results from this study showed that there was significant increase in fiber, protein and total phenolic contents by adding dehydrated broccoli powder. While moisture, ash and crude fat contents showed non-significant effects. Nitrogen free extract showed decreasing trend by increasing broccoli powder in wheat flour. Physical analysis such as loaf volume, specific volume, weight loss, texture showed significantly decreasing effect with the exception of loaf weight. Color analysis and rheological analysis of enriched bread also showed significant effect. The scores assigned to the sensory attributes of broccoli bread including loaf volume, color of crust, color of crumb, grain size, taste, aroma and texture firstly increased upto a level (1% addition), then decreased significantly by increasing concentration of broccoli powder while, scores regarding overall acceptability of bread prepared from composite flour enriched with broccoli powder were 7.08, 7.41, 6.41,5.16 and 3.33(out of 9) for T_0, T_1, T_2, T_3 and T_4 (out of 9). Result concluded that adding broccoli powder higher than 1% resulted in unacceptable textural and sensory properties of bread.

Key words: Broccoli Powder, Bread, Enrichment, proximate, sensory

INTRODUCTION

Wheat is taken as primary source of carbohydrates, proteins, vitamins and minerals (Hoseney *et al.*, 1988). Pakistan is one of the major wheat producing country in the world and contributes 2.1% to the GDP of country (GOP, 2014). Wheat flour is one of the major conventional ingredients in bread making due to its gluten fraction (Mongi *et al.*, 2011). Among cereal baked products, bread plays a significant role to fulfill the dietary needs of people around the world (Cayot, 2007). More than 4000 years ago, man has learned the art of bread making (Cauvain, 2004). The key purpose of bread making is to convert cereal flour into palatable, delicious and digestible food item (Dewettinck *et al.*, 2008). Bread contains high amount of starch and complex carbohydrates that play an important role in the diet of people. The most appealing advantage of the bread is its availability in fresh form round the clock (Rosell *et al.*, 2007). Due to its routine usage and provision of almost 50% of total energy intake, bread can be used as a carrier of healthy ingredients (Akhtar *et al.*, 2009).

Enrichment of bread with natural bio-active components and increase the nutritional value of bread has been the main focus of scientists for many years (Ixtaina *et al.*, 2008). Bread provides a good matrix for enrichment with key nutrients and helps to deliver it to the consumer (Hathorn *et al.*, 2008). However, bread is not considered as a rich source of anti-oxidants, dietary fiber and protein, so vegetables like broccoli are good choice to overcome these deficits (Chlopicka *et al.*, 2012). Vegetables are most important components of our life and their regular use may prevent from different diseases (Temple and Kaiser, 2003). Despite this fact only 30% of adults fulfill the daily recommended intake of vegetables (Hobbs *et al.*, 2014). Broccoli (*Brassica oleracea* L. var. Italica) belongs to the genus Brassica and family Brassicaceae. It is a medicinal therapeutic vegetable and has gained utmost attention due to its health promoting effects in recent years (Ana *et al.*, 2013). According to Mukherjee and Mishra (2012), broccoli is a vegetable crop with significant area under cultivation both in Asia and Europe with great popular graph in United states of America, actually native to Italy and introduced in USA in 1920 by Italian immigrants.

Dietary fibre enriched bakery products are becoming more popular these days due to having more health benefits (Ktenioudak and Gallagher, 2014). Regarding composition of broccoli floret powder, it contains 2.42 g water, 24.57g protein, 5.85g fat, 11.13g dietary fiber, 2.68g ash, 53.62g carbohydrates, 364 kcal energy,

4.65 mg Zn, 0.44mg Cr, 2.56mg K, 6.56mg Fe. However, in fresh form, it contains 89.30% water. It is also a rich source of thiamine, niacin, pantothenic acid, riboflavin, vitamin B6 and selenium (Madhu and Kochhar, 2014). The objectives of the present study were to dehydrate the broccoli and convert into powder and formulation of broccoli enriched bread by adding different proportions of broccoli powder to check its impact on physico-chemical, rheological and sensory attributes of bread.

MATERIAL AND METHODS

Procurement of raw materials

The present research was carried in the Post Graduate Research Laboratory, National Institute of Food Science and Technology, University of Agriculture, Faisalabad. Wheat was purchased from Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan, while bread ingredients and broccoli was procured from reputed stores in Faisalabad. Wheat grains were milled through Brabender Junior Quadrumat Mill for flour.

Dehydration of broccoli

Broccoli florets were washed and cut into pieces of 2cm diameter and blanched for 15 seconds in boiling water. Then blanched broccoli pieces were placed and dried in a conventional dehydrator at 70-75 ºC until dried. These dried pieces were ground in a household grinder to produce a fine powder (Sharma *et al.*, 2011).

Bread preparation

Bread was prepared using the standard formulation given in Table 2 for all the treatments by using standard procedure as outlined in AACC method No. 10-10B (AACC, 2000). Ingredients were mixed for 5-10 minutes in a Hobart A-200 Mixer to form dough and allowed to ferment at 30 °C and 75% R.H. for 180 minutes. First and second punches were made after 120 and 150 minutes, respectively. The dough was molded and panned into 100g test pans, and final proofing was done for 45 minutes at 35 °C and 85% R.H. The bread was baked at 232 °C for 13 minutes.

Analysis of bread

The prepared bread was analyzed for their physical, chemical and organoleptic properties.

Chemical analyses

The bread samples were tested for moisture content by using hot air oven at 105 $\pm$ 5ºC following the procedure described in AACC (2000) method No. 44-15A. The crude protein content in bread samples were determined by Kjeldahl's method as described in AACC (2000) method No. 46-10. The Soxhlet apparatus was used for the determination of crude fat following the AACC (2000) method No. 30-25. The dry fat free bread samples were analyzed for crude fiber content following the procedure mentioned in AACC (2000) method No. 32-10. Each bread sample were analyzed for ash content by following the procedure outlined in AACC (2000) method No. 08-01. The nitrogen free extract was calculated by the following relation:NFE = 100 – (% Moisture+% Crude Protein+% Crude Fat+% Crude Fiber+% Ash Content).

Physical analyses

The color of bread was determined with the help of color meter color test II, serial no. 95808, made in Germany) according to method of Lara *et al*, (2010). Loaf weight of the bread was determined by following the procedure as presented by Das *et al.* (2012). Loaf volume was measured by rapeseed displacement method (Greene and Bovell-Benjamin, 2004). The specific volume of the bread was determined followed by Peressini and Sensidoni (2009).

$$specific\ volume\ c\mathrm{m}^3/\mathrm{g} = \frac{\text{loaf volume of bread}}{\text{weight of bread}}$$

Weight loss

Dough and baked loaf were weighed and percent weight loss calculated followed by Majzoobi *et al.* (2011).

$$\% \text{ Weight loss} = \frac{\text{Weight of raw bread} - \text{Weight of baked bread}}{\text{Weight of bread}} \times 100$$

Texture analysis

Textural analysis of bread was carried out by using Texture Analyzer (TA-XT2 plus Texture Analyzer Stable Micro Systems, serial no. 12028, made in UK) according to method of Piga *et al.*, (2005).

Viscoelastic properties

The viscoelastic properties of wheat and broccoli powder composite flour were recorded by using a Rapid Visco-Analyser RVA-4SA (Newport Scientific Pvt. Ltd, Warned, NSW, Australia) interfaced with a personal computer equipped with thermo cline for windows software. Pasting profile was measured by ACCC method (AACC, 2000). The total phenolic content was determined by Folin-Ciocalteu method (Ashoush and Gadallah, 2011).

Sensory analysis

The experimental breads from different treatments were rated using 9-point hedonic score system (9= like

extremely; 1= dislike extremely) by taste panel. Sensory evaluation performa was made in accordance to Lawless and Heymann, (1998). They were asked to express their opinion about the end product by giving score to attributes like color, flavor, texture, moistness, tenderness, shape and overall acceptability. *During sensorial* evaluation, breads of different treatments were placed in transparent plates, labeled with random codes.

Statistical analysis

The data of each parameter was subjected to statistical analysis to determine the level of significance (Steel *et al.,* 1997).

RESULTS AND DISCUSSION

Proximate analysis of bread

Proximate analysis of each treatment was done and means values are shown in Table 2. Moisture content showed non-significant variation in different treatments. The lowest moisture content was observed in T_3 (32.26%) and maximum in T_4 (34.15%). The non-significant trend is due to lower moisture content present in dried broccoli powder. Similar trend was shown by Das *et al*. (2011) while using broccoli powder. Crude protein contents showed increasing trend significantly by increasing the concentration of broccoli powder as T_4 (14.13%) contained maximum and T_0 (10.20%) contained minimum value for crude protein. The results confirmed the higher amount of protein present in broccoli than wheat flour. Filipčev *et al.* (2015) showed similar findings regarding crude protein. The trend regarding crude fiber showed significantly increasing effect by increasing the concentration of broccoli powder. Maximum value for crude fiber was showed by T_4 (1.61%) and minimum by T_0 (0.80%). Analysis of variance regarding crude fat showed non-significant difference due to the fact that broccoli is low in fat and it has low glycemic index. T_4 showed maximum value (2.31%) and T_0 showed minimum value (2.10%) for crude fat. Similar results for crude fibre and crude fat are reported by Filipčev *et al.,* (2015). The results showed that T_0 has 2.11% ash contents followed by T_1 2.28%, T_2 2.35%, T_3 2.44% and T_4 has 2.51%. It reveals that the ash content of bread increases by increase in broccoli powder level. The mean value of NFE content of different formulated bread was 51.33%, 50.77%, 48.87%, 48.34% and 45.29% of T_0, T_1, T_2, T_3 and T_4 respectively. The results showed that NFE contents decreased with the increased level of dehydrated broccoli powder in bread.

Physical analysis of broccoli enriched bread

Mean values regarding the physical analysis of enriched bread are shown in Table 3. Means regarding loaf volume of enriched bread showed significant decreased in volume by increasing the concentration of broccoli powder as maximum decrease in volume was observed in T_4 (280 cm^3) which contained 7% broccoli and minimum was observed in T_0 (340 cm^3) which was control bread. As broccoli contains higher contents of crude fiber than that of wheat that is why it absorbs more moisture and this ultimately affects the gluten network, and lowers CO_2 gas retention capacity of bread which causes decrease in bread volume. Das *et al.,* (2012) showed the similar trend regarding loaf volume. The analysis of variance regarding the loaf weight showed significant effect by increasing the concentration of broccoli powder, the weight of bread increased. Maximum increase in weight was observed in T_4 (140.51 g) and minimum in T_0 (121.44 g). Tt is due to increase in amount of water absorbed by broccoli powder during dough making and its retention to a large extent in baking process increased loaf weight. Similar trend regarding loaf weight is given by Rakcejeva *et al.,* (2011). Mean values regarding specific volume of broccoli powder composite bread showed significant effect. Specific volume of enriched bread is decreased by increasing broccoli powder concentration. Minimum value for specific volume was exhibited by T_4 (2.09 cm^3/g) and maximum by T_0 (2.83 cm^3/g). Broccoli powder absorbed more moisture that lowers moisture level which effects specific volume of the bread. By increasing the concentration of broccoli powder, weight loss is reduced. Minimum weight loss was observed in T_4 (17.01%) and maximum in T_0 (23.88 %). Weight loss is inversely proportional to the loaf weight, as the broccoli powder enrichment increased bread loaf weight so it resulted in decreased weight loss.

In color analysis, L* value (lightness or luminance) significantly decreased by increasing the broccoli powder concentration. T_0 obtained max. (65.32) and T_4 showed minimum value (50.11) for lightness which shows that bread became darker as broccoli powder concentration is increased as shown by Das *et al.* (2012). a* value (greenish to reddish) showed significantly increasing trend by increasing broccoli powder. T_4 (-1.18) was greener than other 4 treatments and T_0 (-4.32) was least green. By adding dehyderated broccoli powder, b* value (bluish to yellowness) increased non-significantly. Filipčev *et al.* (2015) also showed similar trend for bread color analysis. The analysis of variance regarding the texture of bread showed significant effect and force of

deformation increased by increasing the amount of broccoli powder. Mean values for texture analysis showed maximum value for T_4 (2.53 Kg) and minimum for T_0 (1.52 Kg). Texture depends upon gluten formation and moisture level in bread, as broccoli powder absorbs higher amount of water and deteriorates the gluten network, so the texture became harder (Table 4).

Table 1. Treatment plan

Treatments	Flour (%)	Broccoli Powder (%)
T_0	100	0
T_1	99	1
T_2	97	3
T_3	95	5
T_4	93	7

Table 2. Standard Formulation of Bread

Ingredients	Quantity (g)
Wheat flour	100
Yeast	2
Sugar	5
Salt	2
Shortening	5
Water	According to water absorption capacity

Results regarding viscoelastic properties of broccoli bread showed significant effect on bread as different viscoelastic parameters showed decreasing trend by increasing the broccoli powder concentration. Peak viscosity results showed maximum value for T_0 (9274.4) and minimum in T_4(7261.8). Caballero *et al*. (2008) also showed similar trend for peak viscosity. Break down viscosity showed max. value in T_0 (5052.0) while minimum in T_4 (4170.7). Mean values regarding final viscosity showed significantly decreasing effect by increasing broccoli powder. Max. value was observed in T_0 (8456.8) and min. in T_4 (7002.2). Alamri *et al.,* (2013) reported the similar trend in their research for rheological characteristics. Analysis of variance regarding setback viscosity showed significant effect and showed max. value in T_0 (3309.6) and min. value in T_4 (2283.6). Pasting temperature at which starch granules begin to swell and gelatinize due to water uptake showed non-significant effect. The highest value was (64.48^{o}C) for T_0 and minimum in T_4 (63.07 ^{o}C) (Table 4).

Total phenolic contents (TPC) in bread

Broccoli is a medicinal plant and has considerable quantity of phenolic contents, recognized as a rich source of versatile bio-active compounds such as flavonoids, glucosinolates, phenolic acids etc. (Dziki *et al.,* 2014). Total phenolic contents assay is an accurate index of antioxidant content in bread samples (Kaur and Kapoor, 2002). Table 4 shows that by increasing the concentration of broccoli powder, phenolic contents were also increased. Maximum value for TPC was exhibited by T_4 (66.25mg/100g) with 7% broccoli and minimum value was observed in T_0 (25.03). Findings of TPC are near to the previous findings of Dziki *et al.* (2014).

Sensory evaluation of broccoli powder enriched bread

Results for sensory evaluation of broccoli powder enriched bread are given in Table 5. Mean values regarding crust color of bread showed highest value for T_1 (6.75) and lowest for T_4 (4.08). Crust color affected significantly and became darker by increasing broccoli powder concentration. Dziki *et al.* (2014) reported the similar trend for crust color. Mean values for crumb color of T_1 scored higher (7.41) than other treatments and minimum score obtained by T_4 (3.58). Crumb color became greener as the concentration of broccoli increased in wheat flour bread. The grain size referred to the structure of crumb which greatly affected by the addition of broccoli powder by increasing concentration grain size become irregular. The highest score was achieved by T_1 (7.41) and lowest by T_4 (4.10). Taste is the main character that greatly influences the consumer's choice. Maximum score for taste was obtained by T_1 (7.50) and minimum by T_4 (3.41). Broccoli is somewhat bitter in taste so by increasing its amount it starts producing off taste. Mean values regarding bread texture showed significant differences by adding broccoli powder. The maximum score was achieved by T_1 (7.33) and minimum by T_4 (5.08). Aroma is also an important property of bread which influences the consumer's acceptance of food. Means values for aroma showed significant effect as by increasing broccoli concentration aroma became un-pleasant. T_0 obtained maximum scores (7.33) while T_4 obtained minimum score (3.58) for aroma. Bread Volume mainly depends on gluten network formation and moisture level. By

Table 3. Proximate analysis of bread

Treatments	Moisture %	Protein %	Fiber %	Fat %	Ash %	NFE %
T_0	33.46±1.67	10.20±0.51	0.80±0.04	2.10±0.11	2.11±0.08	51.33±0.54
T_1	32.52±1.61	11.35±0.48	0.94±0.06	2.14±0.15	2.28±0.17	50.77±1.07
T_2	33.89±1.63	11.98±0.56	1.24±0.05	2.20±0.17	2.35±0.36	48.87±0.51
T_3	32.26±1.59	12.85±0.39	1.31±0.07	2.27±0.22	2.44±0.55	48.34±0.71
T_4	34.15±1.56	14.13±1.56	1.61±0.04	2.31±0.26	2.51±0.73	45.29±0.85

Table 3. Physical analysis of bread

Treatment	Color			Loaf volume (cm^3)	Specific volume (cm^3/g)	Loaf weight (g)	Weight loss (%)
	L*	a*	b*				
T_0	65.32±3.27	-4.32±0.22	26.42±1.32	340.22±6.63	2.83±0.14	121.44±6.05	23.88±0.98
T_1	63.62±3.18	-3.91±0.20	27.09±1.35	315.42±4.44	2.55±0.13	126.23±6.3	22.37±0.89
T_2	60.42±3.02	-2.34±0.12	28.30±1.42	311.33±5.51	2.26±0.12	132.13±5.21	19.88±0.79
T_3	54.48±2.72	-1.40±0.07	29.11±1.42	303.11±6.81	2.18±0.11	137.21±4.81	17.22±0.80
T_4	50.11±1.44	-1.18±0.06	31.12±1.33	280.21±5.91	2.08±0.11	140.51±5.11	17.01±0.76

Table 4. Texture, TPC and Rheological analysis of bread

Treatments	Texture (Kg)	TPC (mg/100g)	Peak viscosity	Break down viscosity	Final viscosity	Setback viscosity	Pasting temperature (ºC)
T_0	1.46±0.05	25.03±0.08	9274.41±201.22	5025.01±100.32	8456.81±174.71	3309.6±71.3	64.4±1.4
T_1	1.52±0.04	35.74±0.09	8439.71±197.34	4723.51±95.44	7949.41±169.77	2879.3±66.3	63.8±1.1
T_2	1.63±0.03	47.24±0.04	8068.73±128.13	4472.21±66.23	7678.71±118.34	2581.5±59.1	63.7±1.3
T_3	2.12±0.16	59.74±0.10	7697.71±117.64	4271.21±54.41	7306.61±113.12	2409.3±44.3	63.4±0.9
T_4	2.53±0.11	66.25±0.07	7261.81±113.22	4170.71±44.44	7002.21±139.31	2283.6±73.5	63.0±1.0

Table 5. Sensory evaluation of bread

Treatments	Crust color	Crumb color	Grain size	Taste	Aroma	Loaf volume	Overall acceptability
T_0	6.75±o.25	7.41±0.38	7.16±0.28	7.16±0.28	7.33±0.38	7.08±0.14	7.08±0.14
T_1	6.91±0.38	7.25±0.25	7.41±0.38	7.50±0.25	7.25±0.25	6.91±0.25	7.41±0.38
T_2	5.16±0.28	6.16±0.38	6.20±0.34	5.91±0.14	6.08±0.38	6.66±0.38	6.41±0.52
T_3	4.25±0.25	5.25±0.50	5.50±0.50	4.66±0.57	4.83±0.76	5.41±0.62	5.16±0.28
T_4	4.08±0.14	3.58±0.52	4.91±0.14	3.41±0.52	3.58±0.52	3.33±0.57	3.33±0.57

increasing the concentration of broccoli, more deterioration of gluten network was observed and more water was bound which ultimately disturbed the volume. T_0 was ranked with highest score (7.08) while T_4 (3.33) obtained minimum score. For overall acceptability, bread enriched with 1% broccoli powder (T_1) ranked first and bread with 3% broccoli powder (T_2) was somehow acceptable but other two breads with the addition of 5% (T_3) and 7% (T_4) broccoli powder were found unacceptable.

CONCLUSION

Broccoli is a good source of nutrition and considered as a medicinal plant and jewel of nutrition. It is rich

source of dietary fiber, protein and phenolic contents. Bread provides a good matrix for enrichment. Broccoli is added to bread due to its unique nutritional properties. In present study, it was added in different proportions (1%, 3%, 5% and 7%). The bread prepared with 1% broccoli powder showed better characters than supplemented with 3, 5 and 7% broccoli powder. The addition of broccoli powder increases the protein, fiber and total phenolic contents of bread but negatively affected the rheological and some physical parameters. Addition of broccoli in bread can be used to increases the nutritional value of bread and by this way vegetable consumption can be improved.

Acknowledgment

We are thankful to National Institute of Food Science and Technology, Faculty of Food, Nutrition and Home Sciences, University of Agriculture, Faisalabad for providing best facilities for conducting this research.

Conflict of interest

The authors have no conflict of interest.

REFERENCES

1. AACC. 2000. Approved methods of American Association of Cereal Chemists, 10th edition American Association of Cereal Chemists, Incorporation Saint Paul, MN, USA.
2. Akhtar, S., F.M. Anjum and S.U. Rehman. 2005. Effect of iron and zinc fortification on chemical composition of whole wheat flour. J. Res. Sci. 16:95-103.
3. Alamri, M.S., A.A. Mohamed and S. Hussain. 2013. Effects of alkaline-soluble okra gum on rheological and thermal properties of systems with wheat or corn starch. Food Hydrocolloid. 30: 541-551.
4. Ana, M. A., J. N. Maria and J. Bernal. 2013. Extraction, chemical characterization and biological activity determination of broccoli health promoting compounds. J. Chromatography A. 1313: 78-95.
5. Ashoush, I.S. and M.G.E. Gadallah. 2011. Utilization of mango peels and seed kernels powders as sources of phytochemicals in biscuit. World J. Dairy Food Sci. 6: 35-42.
6. Caballero, P.A., M. Gomez.and C.M. Rosell. 2007. Improvement of dough rheology, bread quality and bread shelf-life by enzymes combination. Food Engr. 81: 42-53.
7. Cauvain, S.P. 2004. How much more bread research do we need? Getreide Technol. 58: 364-366
8. Cayot, N. 2007.Sensory quality of traditional foods. Food Chem. 102: 445-453.
9. Chlopicka, J., P. Pasko, S. Gorinstein, A. Jedryas, and P. Zagrodzki. 2012. Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads. LWT- Food Sci. Technol. 4: 548-555.
10. Das, L., U. Raychaudhuri and R. Chakraborty. 2012. Supplementation of common white bread by coriander leaf powder. Food Sci. Biotechnol. 21: 425-433.
11. Dewettinck, K., F.V. Dockstaele, B. Kuhne, D.V. Walle, T.M. Courtens and X. Gellynck. 2008. Nutritional value of bread: Influence of processing, food interaction and consumer perception. J. Cereal Sci. 48: 243-257.
12. Dziki, U.G., M. Swieca, D. Dziki, L. Seczyk, U. Zlotek, R. Royylo, K. Kaszuba, D. Ryszawy and J. Czyz. 2014. Anticancer and antioxidant activity of bread enriched with broccoli sprouts. BioMed Res. Int. 1: 1-14.
13. Filipčev, B., L. Levic. M.B. Solarov, N. Misljenovic and G. Kopprivica. 2015. Quality characteristic and antioxidant properties of breads supplemented with Sugar beet molasses of beet. Int. J. Food Proper. 13: 1035-1053.
14. GOP (Government of Pakistan). 2015. Economic survey of Pakistan. Economic affairs division, ministry of finance, Islamabad, Pakistan.
15. Greene, J.L. and A.C.B. Benjamin. 2004. Macroscopic and sensory evaluation of bread supplemented with sweet potato flour. J. Food Sci. 69: 167-173.
16. Hathorn, C. S., M. A. Biswas, P. N. Gichuhi and A. C. B. Benjamin. 2008. Comparison of chemical, physical, micro-structural, and microbial properties of breads supplemented with sweet potato flour and high-gluten dough enhancers. Food Sci. Technol. 41: 803-815.
17. Hobbs, D. A., A. Ashouri, T. W. George, J. A. Lovegrove & L. Methven. 2014. The consumer acceptance of novel vegetable-enriched bread products as a potential vehicle to increase vegetable consumption. Food Res. Intl. 58:15-22.
18. Hoseney, R.C., P. Wade and J.W. Finley. 1988. Soft wheat products. Wheat chem. Technol. 2:. 407- 456.
19. Ixtaina, V.Y., S.M. Nolasco and M.C. Tomas. 2008. Physical properties of chia (*Salvia hispanica* L.) seeds. Ind. Crops Prod. 28: 286-293.
20. Kaur, C. and H.C. Kapoor. 2002. Anti-oxidant activity and total phenolic content of some Asian vegetables. Int. J. Food Sci. Technol. 37: 158-161.
21. Ktenioudak, A. and E. Gallagher. 2014. Recent advances in the development of high-fibre baked products. Trends Food Sci. Technol. 28: 4-14.
22. Lara, E., P. Cortes, V. Briones and M. Perez. 2011. Structural and physical modifications of corn biscuits during baking process. Food Sci. Technol. 44: 622-630.
23. Lawless, H.T. and H. Heymann. 1998. Acceptance and Preference Testing. Sensory Evaluation Food. 6: 430-475.
24. Madhu and A. Kochhar. 2014. Proximate composition, available carbohydrates, dietary fiber and anti-nutritional factors of broccoli

(*Brassica oleraceal* var. Italicaplenca) leaf and floret powder. Biosci. Discovery. 5: 45-49.
25. Majzoobi, M., A. Farahnaky and S. Agah. 2011. Properties and shelf-life of part-and full-baked flat bread (Barbari) at ambient and frozen storage. J. Agric. Sci. Technol. 13: 1077-1090.
26. Mongi, R.j., B.k. Ndabikunze, B.E. Chove, P. Mamiro, C.C. Ruhembe and J.G. Ntwenya. 2011. Proximate composition, bread characteristics and sensory evaluation of cocoyam-wheat composite breads. African J. Food Agri. Nutri. Develop. 11(7): 5586-5599.
27. Mukherjee, V. and P.K. Mishra. 2012. Broccoli-an underexploited netraceutical. Sci. Res. Reporter. 2:291-294.
28. Peressini, D. and A. Sensidoni. 2009. Effect of soluble dietary fibre addition on rheological and bread making properties of wheat doughs. J. Cereal Sci. 49: 190-201.
29. Piga, A., P. Catzeddu, S. Farris, T. Roggio, A. Sanguinetti and E. Scano. 2005. Texture evaluation of "Amaretti" biscuits during storage. Eur. Food Res. Technol. 22: 387-391.
30. Rakcejeva,T., R. Galoburda, L. Cude and E. Strautniece. 2011. Use of dried pumpkins in wheat bread production. Procedia Food Sci. 1: 441-447.
31. Rosell, R., M. Skrzypski, E. Jaseem, M. Taron, R. Bartolucci, J. J. Sanchez, P. Mandez, I. Chaib, L. Perez-r-Roca, A. Szymanowska, W. Rzyman, F. Puma, G. K. Guilda, R. Farabi and J. Jassem. 2007. A novel prognostic factor in resected non-small-cell lung cancer. DOI: 10.1371/journal. pone. 0001129.
32. Sharma, K.D., K. Stahler, B. Smith and L. Melton. 2011. Antioxidant capacity, polyphenolics and pigments of broccoli-cheese powder blends. J. Food Sci. Technol. 48: 510-514.
33. Steel, R.G.D., J.H. Torrie and D.A. Dicky. 1997. Principles and Procedures of statistics: A biometrical approach. 3rd Edition McGraw Hill Book Co. Inc., NY, USA.
34. Temple, N.J. and G.K. Kaiser. 2003. Fruit, vegetables, and the prevention of cancer. Res. challenges. Nutri. 19: 467-470.

PAK. J. FOOD SCI., 27(1), 2017: 46-52
ISSN: 2226-5899

Pathophysiological role of leptin for human health: A review

Fasiha Ahsan*, Mian Kamran Sharif, Masood Sadiq Butt, Aamir Shehzad and Muhammad Issa Khan

National Institute of Food Science and Technology, Faculty of Food, Nutrition and Home Sciences, University of Agriculture Faisalabad, Pakistan

***Corresponding author email:** fasiha23.fa@gmail.com

ABSTRACT

Leptin, a protein secreted from fatty tissue, is believed to control fat accumulation in the body. It is secreted in pulsatile fashion like other hormones and its secretion rate depends on adipose tissues. Leptin is not the obesity but a starvation hormone. It works in body by binding with receptors called *ob* and stimulates the different signal transduction pathways especially those which contain these receptors. Defects in leptin receptors (*db/db*) or in leptin genes (*ob/ob*) may results in numerous metabolic abnormalities in human body and disrupt the normal physiology of body. Appropriate availability of leptin in adipose tissues plays much important role in regulating body functions like energy homeostasis, neuroendocrine function, insulin resistance and metabolic syndrome. Leptin responds differently with high and low energy states in body. Leptin receptors present on hypothalamus stimulate the nervous system to ensure energy homeostasis. Low levels of leptin affect the secretions of reproductive and thyroid hormones further causing complications in pregnancy and metabolic activities. Results from interventional studies in humans demonstrate that leptin administration in subjects with congenital leptin deficiency, lipoatrophy and women with hypothalamic amenorrhea reverses the energy homeostasis and neuroendocrine and metabolic abnormalities associated with these conditions. Furthermore, disturbance in leptin receptors or gene may contribute resistance in insulin functioning and metabolic syndromes. The stated functions can be managed by ensuring optimum levels of leptin in human body.

Key words: Leptin, obesity, energy homeostasis, insulin resistance, metabolic syndromes, neuroendocrine function,

INTRODUCTION

Leptin is derived from the Greek word "*Leptos*" meaning "Thin". Its discovery has created great enthusiasm due to possible treatment of obesity. Generally adipose tissues are considered only meant for energy storage; however, recent studies have proven it as active endocrine organ. Initial clinical trials of leptin have proven it an ineffective strategy for obesity treatment (Heymsfield *et al.,* 1999; Sendlhofer *et al.,* 2015). However, scientists have undertaken extensive researches on leptin to explain its role in human physiology. These studies are more helpful for understanding leptin role in in energy homeostasis, insulin resistance and regulation of neuroendocrine function mainly in obesity state. Leptin is composed of total 167 amino acids and it was primarily identified from the positional cloning of mice gene called *ob/ob* at Jackson Laboratories (Zhang *et al.,* 1994). Proper examination of these mice has shown mutation in their leptin gene and this alteration was homozygous resulting in deficiency of leptin which cause the conditions like diabetes, abnormalities in neuro-endocrine functions, infertility and hyper-phagia. Adipose tissues are responsible for its synthesis (Considine *et al.,* 1996) and it is secreted in a pulsatile fashion like other hormones. The level of leptin is higher in the early morning hours and in evening (Sinha *et al.,*1996; Licinio *et al.,*1997). The presence of leptin in blood circulation reflects alterations in caloric ingestion and consequently higher energy storage in fatty tissues (Boden *et al.,* 1996; Chan *et al.,* 2003; Chan *et al.,* 2008).

Function of leptin starts in body when it binds to specific receptors called *Ob* receptors which are present in peripheral tissues and brain (Bjorbaek *et al.,*1998). After binding with receptors, it activates many signal transduction pathways, including pathways of Phosphatidylinositol 3 kinase (PI3K), Janus Kinase Signal Transducer and Activator of Transcription 3 (JAK-STAT3). PI3K is essential for glucose homeostasis and food ingestion (Niswender *et al.,*2001), JAK-STAT3 pathway, it is important for the proper homeostasis of energy (Bates *et al.,* 2003). Likewise, additional pathways Mammalian Target of Rapamycin (mTOR), Adenosine Monophosphate Protein Kinase (AMPK) and Mitogen Activated Protein Kinase (MAPK) are currently under study (Robertson *et al.,* 2008). Hypothalamic mechanisms involved in the leptin resistance include a) mutation in *Ob* receptors, b) induce the response of leptin signaling and c) changes in the passage of leptin through the blood-brain barrier (Bjorbaek *et al.,*1999; Myers *et al.,* 2008; Munzberg, 2008). However, further studies are required to fully understand the role of leptin in different signaling pathways which in turn may provide some useful strategies in the treatment of obesity.

Role in human physiology

Leptin plays critical role in energy homeostasis, metabolism and regulation of neuroendocrine functions (Chan *et al.,* 2005; Park and Ahima, 2015) which are discussed below:

Energy homeostasis

Leptin circulation in body helps to measure the amount of stored energy and guides the nervous system to maintain the ingestion of food and expenditure of energy accordingly. It has the immediate effect on brain for appetite regulation and exerts its action by binding with *Ob* receptors in hypothalamic part of brain (Fig 1).

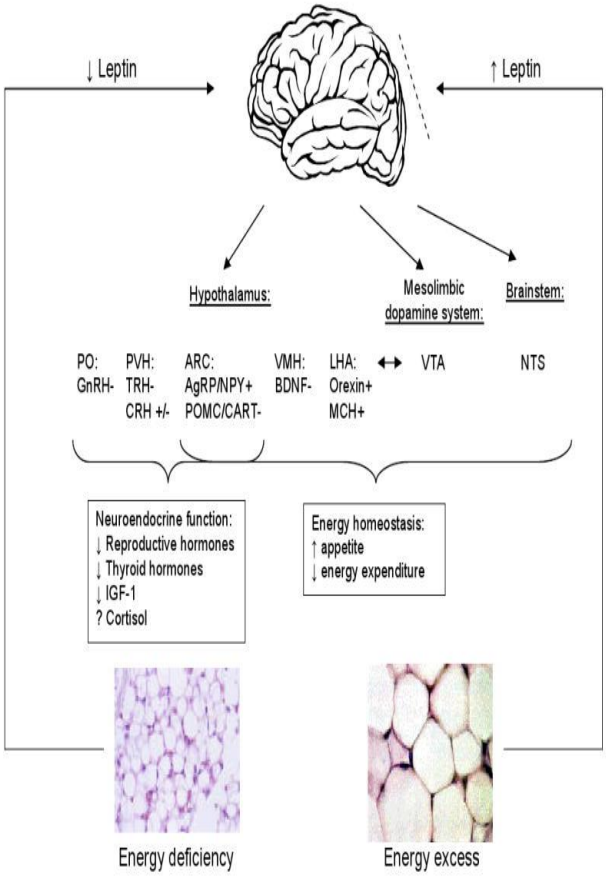

Figure 1. Regulation of energy homeostasis via leptin

(Adopted from: Kelesidis *et al.*, 2010)

Leptin responds differently with high and low energy states in body. High energy states are related with high level of leptin. Consequently, in this case appetite inhibiting neuropeptides: Cocaine and Amphetamine regulated Transcript (CART) and Proopiomelanocortin (POMC) reduced in ARC (Cowley *et al.,* 2001) also Brain Derived Neurotrophic Factor (BDNF) in the ventromedial hypothalamic nucleus (VMH). In addition to this leptin also perform its action by acting on ventral tegmental area (VTA) of system called dopamine to adjust stimulus and incentive of food intake. In brainstem, it stimulates the activity of NTS which involve in the control of satiety. In contrary to high energy states, lack of energy is responsible for low level of leptin in human body. Consequently, a complex neural circuit with the help of orexigenic signals triggered the intake of food (Robertson *et al.,* 2008). Due to this process the appetite stimulating neuropeptides (orexigenic): Neuropeptide Y (NPY) and Agouti related Protein (AgRP) increased their expression in arcuate nucleus (ARC) (Cowley *et al.,* 2001) and also Melanin concentrating Hormone (MCH) and orexin in lateral hypothalamic area (LHA). Leptin also showed its direct effects on preoptic area (PO) and paraventricular nucleus (PVN) which are essential for neuroendocrine functions in response to less availability of energy, conditions including reducing thyroid and reproductive hormones. The indirect effect of leptin only on gonadotropin releasing hormone (GnRH), its effect on thyrotropin releasing hormone (TRH) and corticotropin releasing hormone (CRH) may be direct or indirect (Robertson *et al.,* 2008). Its outcome on hormone cortisol during less energy varies in human and mice. Not like normal mice (Heiman *et al.,* 1997), replacement dosage of leptin cannot inverse the raised adreno corticotropin (ACTH) levels related with hunger in humans (Robertson *et al.,* 2008).

Leptin has the ability to cross the blood-brain-barrier in order to reach in hypothalamus where it stimulates the complex neural system. This system is composed of neuropeptides that control the intake of food called orexigenic (stimulate the appetite) and anorexigenic (inhibit the appetite). Leptin also perform its role outside of the hypothalamic part of brain, where it interacts with dopamine, which actually intricate the motivation and reward of feeding and to the nucleus of the solitary tract (NTS) that contribute to satiety (Robertson *et al.,* 2008). In addition to its instant effects, the persistent usage of leptin may result in the connections rewiring between the hypothalamic neurons (Bouret *et al,* 2004; Pinto *et al.,* 2004). Especially in leptin deficient mice, the long-term administration has been exposed to induce quantities of synapses on neurons (anorexigenic) which are involved in the secretion of pro-opio-melano-cortin (POMC) and reduce the synapses number on neuro-peptide-Y (NPY) which are orexigenic (Pinto *et al.,* 2004). Role of leptin not just to give signals to brain for food intake but it also perform its function in energy expenditure. It activates the sympathetic activity and stimulates the thermogenesis in brown adipose tissue in mice (although these effects of leptin have not been established in human beings (Collins *et al.,* 1996; Haynes *et* al., 1997; Chan *et al.,* 2007). Clinical trials have shown that patients with inherited deficiency of leptin due to alteration in its gene or in leptin receptor are obese specifically with the presence of hyper-phagia (Strobel *et al.,* 1998; Farooqi *et al.,* 2007), and when these patients received the replacement dosage of leptin then they attained the normal body weight (Farooqi *et al.*,2007).

Regulation of neuroendocrine functions

In case of any changes in the proportion of body fat mass, leptin stimulates the activity of neuroendocrine toward short-term deficit of energy (Ahima *et al.,* 1996; Chan *et al.,* 2003; Chan *et al.,* 2006). Resultantly leptin lowers the secretion of reproductive and thyroid hormone, causing complications in pregnancy and decrease the metabolic activities respectively. However, it increased the level of growth hormone which mobilize the energy storage in human and mice (Ahima *et al.,* 1996; Chan *et al.,* 2003; Chan *et al.,* 2008). The interactions of leptin with adrenal axes and growth hormone are not so much important in human as compared to the mice. In patients with inherited deficiency of leptin, normal functioning of adrenal glands has been observed (Farooqi *et al.,* 2002; Ozata *et al.,* 1999). Numerous Studies have shown defective neuroendocrine function in prolonged period of starvation (Chan *et al.,* 2003). In another study, leptin deficiency was induced in women with normal weight and higher levels of baseline leptin, there was decrease in leptin levels up to 2.8 nanogram per milliliter (Chan *et al.,* 2006). Later on it was revealed that leptin threshold of approximately 3 nanogram per milliliter is needed to send the messages to brain that stores of energy in fatty tissues are sufficient to bring pregnancy to term. Leptin level greater than 3 nanogram per milliliter in children allows the puberty onset and in old age persons sustains the neuroendocrine functions (Mantzoros *et al.,* 1997). The conditions like anorexia nervosa and amenorrhea (Chan and Mantzoros, 2005) were related with low leptin level or hypoleptinemia in women who were chronically deficient in energy. This hypothesis was confirmed by observational studies (Mantzoros *et al.,* 1997; Audi *et al.,* 1998; Miller *et al.,* 1998). Low leptin levels are assossiated with neuroendocrine abnormalities following osteoporosis. The study results have shown that when replacement dosage of leptin was given to amenorrhea women, it completely regularized the thyroid, gonadal and less degree of bone markers along with growth hormone.

Insulin resistance and metabolic syndrome

It has been confirmed from previous studies that if there is any disturbance in leptin receptors (*db/db*) or leptin gene (*ob/ob*) present in both mice and human may result resistance in insulin functioning (Fig 2) and metabolic syndrome. In *ob/ob* mice improvement in insulin and glucose levels prior to optimum weight loss is achieved with leptin adminstration (Harris *et al.,* 1998). In a study leptin treatment given to the patients showed significant improvements in high insulin levels and HDL besides decrease in LDL and triglycerides (Farooqi *et al.,* 2002). Studies regarding lipoatrophy (lack of subcutaneous fat) in mouse models indicate that these models have low level of leptin due to less fatty tissues, which are necessary for leptin production. In such models metabolic anomalies like insulin resistance, high level of lipid and glucose are commonly present (Gavrilova *et al.,* 2000). When the replacement dose of leptin was provided to these lipoatrophic models a positive response towards exogenous leptin was exhibited (Shimomura *et al.,* 1999). Later it was observed that, if adipose tissues are transplanted to these mice (Gavrilova *et al.,* 2000; Kim *et al.,* 2002), which have ability to produce leptin as well as administrated exogenous leptin (Shimomura *et al.,* 1999) it will definitely improve the conditions like hepatic steatosis, insulin resistance, hyperlipidemia and hyperglycemia (Javor *et al.,* 2002; Oral *et al.,* 2002; Ebihara *et al.,* 2004).

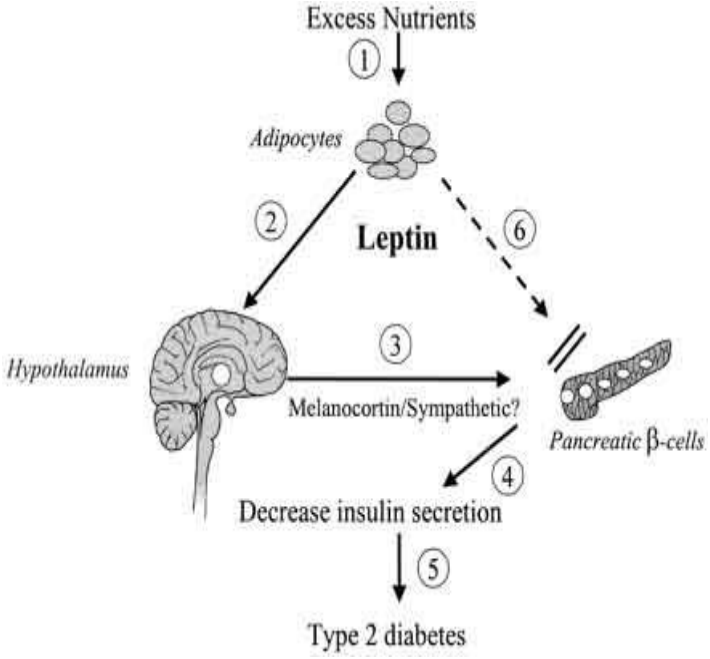

Fig. 2. Leptin and insulin resistance

Leptin therapy in human disease

Leptin therapy has been observed in several leptin deficiency conditions. The main conditions which have been studied are congenital leptin deficiency, lipodystrophy and hypothalamic amenorrhea (Chan and Mantzoros, 2005; Kelesidis and Mantzoros, 2006).

Congenital leptin deficiency

It is a rare autosomal recessive disease triggered by alterations in the leptin gene and associated with insufficient release of Gonadotropin Releasing Hormone (GnRH), revealing the symptoms of hypogonadism, absence of growth spurt, improper secondary sex

characteristics and disturbances in menstruation (Tsiodras *et al.,* 2010).With the proper dosage of Leptin many symptoms can be reversed which occurred by congenital leptin deficiency, *e.g.* there is a significant weight loss in five adult patients which were treated with leptin, the average body mass index fall from 51.5 to 29.2 kg/m^2 (Paz-Filho *et al.,* 2010). It can also improve the symptoms of dyslipidemia and hyperinsulinemia in these individuals (Gibson *et al.,* 2004). Leptin replacement also be helpful in neuro-endocrine function with significant improvements in puberty (Farooqi *et al.,* 2002; Strobel *et al.,* 2003; Licinio *et al.,* 2004). In a study, proper administration of leptin in three subjects with congenital leptin deficiency revealed, a steady rise in gonadotropins level and normalize the release of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) after 24 months of leptin therapy (Strobel *et al.,* 1998). It was also observed in adults that leptin therapy enhanced the testosterone level, axillary hair production and sperm production (Licinio *et al.,* 2004). Leptin also perform its role in immune function, patients with congenital leptin deficiency have a greater prevalence of infection than the normal individuals, reason is to decreased production and functionality of CD4+ T cells, which normalizes with proper leptin therapy. Moreover, Amylin compassionate leptin access program is use for the long-term treatment of the subjects with leptin deficiency (Mantzoros, 2010; Fiorenza *et al.,* 2011).

Lipodystrophy

It is an adipose tissue disorder with decreased the rate of subcutaneous adipose tissues and increase in visceral adipose tissue. Lipodystrophy is a autosomal recessive disorder, related with consanguineous marriage (Nishiyama *et al.,* 2009). Exogenous Leptin administration has been observed in nearly 100 subjects with this disorder. The results of these studies revealed that leptin therapy significantly improves insulin sensitivity and dyslipidemia in these subjects and decreases hepatic gluconeogenesis, glycosylated hemoglobin and intrahepatic fat content (Petersen *et al.,* 2002; Javor *et al.,* 2005). Additionally, many studies in humans and rats have exhibited that leptin therapy has its direct effect glucose metabolism enhancement and activating the signaling pathways (Brennan and Mantzoros, 2006) in metabolically important tissues (muscles), but these pathways are not much similar with the normal pathways for insulin activation (Moon *et al.,* 2011). Most prevalent condition of this disorder is HIV-associated lipoatrophy, affecting approximately 14.9 to 35.6% of HIV infected subjects and is also linked with abnormality in metabolism of adipose tissues and insulin resistance (Tsiodras and Mantzoros, 2006; Sekhar *et al.,* 2011). In a study, randomized double blind, interventional study, we observed that leptin in significant dosage improves hyperlipidemia, truncal fat mass and insulin resistance in subjects (Lee *et al.,* 2006). It was also observed from the study that demonstration of leptin in HIV lipoatrophic subjects which were taken pioglitazone also improves glucose metabolism (Magkos *et al.,* 2011). The promising effects of leptin on glucose metabolism in these subjects could be due to strong effect of pioglitazone (a thiazolidinedione) on adiponectin secretion and plasma concentration because leptin therapy alone could not cause alterations in adiponectin levels (Gavrila *et al.,* 2005).

Hypothalamic amenorrhea

It is a condition of infertility and major cause of absent menstrual periods and usually seen in hypoleptinemic women who are in very low energy state, *e.g.* who suffer from anorexia nervosa and who exercise vigorously (Bluher *et al.,* 2009; Kelesidis *et al.,* 2010). The promising treatment for infertility in such women is Leptin replacement therapy (Bluher and Mantzoros, 2004). In a study, proper administration of leptin showed, an improvement in the release of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) after 24 months of leptin therapy (Welt *et al.,* 2004). Results of another nine-month randomized, double blind and placebo controlled study of leptin therapy revealed (Chou *et al.,* 2011). That there was continuation of menstrual periods in approximately 70% of the women, while nearly 60% of them who menstruated were enter in the phase of ovulation. Most Importantly, increase in the dose of leptin in women which were not menstruate due to less leptin level turned to menstruate and resolve the amenorrhea. Moreover, ten weeks administration of leptin also useful in increasing the bone forming markers in women with hypothalamic amenorrhea, but study results shown no alterations
in in total and regional bone mass density, which was not shocking because the short period of study (Welt *et al.,*2004).

Conclusion

Leptin is not the obesity but a starvation hormone it plays a fundamental role in some important body functions including energy homeostasis, regulation of neuroendocrine function, insulin resistance and metabolism, not just in surplus energy conditions but, most importantly, in the situations of insufficient energy and starvation. The stated functions can be managed by ensuring optimum levels of leptin in human body. Randomized, controlled clinical trials were proved that proper leptin administration has potential to correct the abnormalities such as congenital leptin deficiency, lipodystrophy and hypothalamic amenorrhea, and also

play important role in the development of leptin sensitizers for common obesity.

REFERENCES

1. Ahima, R.S., D. Prabakaran, C. Mantzoros, D. Qu, B. Lowell and E. Maratos-Flier. 1996. Role of leptin in the neuroendocrine response to fasting. J. Nat. 382: 250–252.
2. Audi, L., C.S. Mantzoros, A. Vidal-Puig, D. Vargas, M. Gussinye and A. Carrascosa. 1998. Leptin in relation to resumption of menses in women with anorexia nervosa. Mol. Psychiatry. 3: 544–547.
3. Bates, S.H., W.H. Stearns, T.A. Dundon, M. Schubert, A.W. Tso and Y. Wang. 2003. STAT3 signalling is required for leptin regulation of energy balance but not reproduction. J. Nat. 421: 856–859.
4. Bjorbaek, C., J.K. Elmquist, P. Michl, R.S. Ahima, A. van Bueren and A.L. McCall. 1998. Expression of leptin receptor isoforms in rat brain microvessels. J. Endocrinol. 139: 3485–3491.
5. Bjorbaek, C., K. El-Haschimi, J.D. Frantz and J.S. Flier. 1999. The role of SOCS-3 in leptin signaling and leptin resistance. J. Biol. Chem. 274: 30059–65.
6. Bluher, S. and C.S. Mantzoros. 2004. The role of leptin in regulating neuroendocrine function in humans. J. Nutr. 134: 2469S–2474S.
7. Bluher, S., S. Shah and C.S. Mantzoros. 2009. Leptin deficiency: clinical implications and opportunities for therapeutic interventions. J. Investig. Med. 57: 784–788.
8. Boden, G., X. Chen, M. Mozzoli and I. Ryan. 1996. Effect of fasting on serum leptin in normal human subjects. J. Clin. Endocrinol. Metab. 81:3419–3423.
9. Bouret, S.G., S.J. Draper and R.B. Simerly. 2004. Trophic action of leptin on hypothalamic neurons that regulate feeding. J. Sci. 304: 108–110.
10. Brennan, A.M. and C.S. Mantzoros. 2006. Drug Insight: the role of leptin in human physiology and pathophysiology—emerging clinical applications. Nat. Clin. Pract. Endocrinol, Metab. 2: 318–327.
11. Chan, J.L. and C.S. Mantzoros. 2005. Role of leptin in energy-deprivation states: normal human physiology and clinical implications for hypothalamic amenorrhoea and anorexia nervosa. Lancet. 366: 74–85.
12. Chan, J.L., C.J. Williams, P. Raciti, J. Blakeman, T. Kelesidis and I. Kelesidis. 2008. Leptin does not mediate short-term fasting-induced changes in growth hormone pulsatility but increases IGF-I in leptin deficiency states. J. Clin. Endocrinol. Metab. 93: 2819–2827.
13. Chan, J.L., G. Matarese, G.K. Shetty, P. Raciti, I. Kelesidis and D. Aufiero. 2006. Differential regulation of metabolic, neuroendocrine, and immune function by leptin in humans. Proc. Natl. Acad. Sci. 103: 8481–8486.
14. Chan, J.L., J. Bullen, V. Stoyneva, A.M. Depaoli, C. Addy and C.S. Mantzoros. 2005. Recombinant methionyl human leptin administration to achieve high physiologic or pharmacologic leptin levels does not alter circulating inflammatory marker levels in humans with leptin sufficiency or excess. J. Clin. Endocrinol. Metab. 90: 1618–1624.
15. Chan, J.L., J.E. Mietus, P.M. Raciti, A.L. Goldberger and C.S. Mantzoros. 2007. Short-term fasting-induced autonomic activation and changes in catecholamine levels are not mediated by changes in leptin levels in healthy humans. Clin. Endocrinol. 66: 49–57.
16. Chan, J.L., K. Heist, A.M. DePaoli, J.D. Veldhuis and C.S. Mantzoros. 2003. The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. J. Clin. Invest. 111: 1409–1421.
17. Chou, S.H., J.P. Chamberland, X. Liu, C. Gao, H. Gong and C.S. Mantzoros. 2011. Leptin is an effective treatment for hypothalamic amenorrhea. Proc. Natl. Acad. Sci. USA. 108: 6585–6590.
18. Collins, S., C.M. Kuhn, A.E. Petro, A.G. Swick, B.A. Chrunyk and R.S. Surwit RS. 1996. Role of leptin in fat regulation. J. Nat. 380: 677.
19. Considine, R.V., M.K. Sinha, M.L. Heiman, A. Kriauciunas, T.W. Stephens and M.R. Nyce. 1996. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. 334: 292–295.
20. Cowley, M.A., J.L. Smart, M. Rubinstein, M.G. Cerdan, S. Diano and T.L. Horvath. 2001. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. J. Nat. 411: 480–484.
21. Ebihara, K., H. Masuzaki and K. Nakao. 2004. Long-term leptin-replacement therapy for lipoatrophic diabetes. N. Engl. J. Med. 351: 615–616.
22. Farooqi, I.S., E. Bullmore, J. Keogh, J. Gillard, S. O'Rahilly and P.C. Fletcher. 2007. Leptin regulates striatal regions and human eating behavior. J. Sci. 317: 1355.
23. Farooqi, I.S., G. Matarese, G.M. Lord, J.M. Keogh, E. Lawrence and C. Agwu. 2002. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J. Clin. Invest.110: 1093–1103.
24. Farooqi, I.S., T. Wangensteen, S. Collins, W. Kimber, G. Matarese and J.M. Keogh JM. 2007. Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. N. Engl. J. Med. 356: 237–247.
25. Farooqi, I.S., G. Matarese, G.M. Lord, A.M. DePaoli and S. O'Rahilly. 2002. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J. Clin. Invest. 110: 1093–1103.
26. Fiorenza, C.G., S.H. Chou and C.S. Mantzoros. 2011.Lipodystrophy: pathophysiology and advances in treatment. Nat. Rev. Endocrinol 7: 137–150.
27. Gavrila, A., W. Hsu, S. Tsiodras and C.S. Mantzoros. 2005. Improvement in highly active antiretroviral

therapy-induced metabolic syndrome by treatment with pioglitazone but not with fenofibrate: a 2 × 2 factorial, randomized, double-blinded, placebo-controlled trial. Clin. Infect. Dis. 40: 745–749.
28. Gavrilova, O., B. Marcus-Samuels, D. Graham, J.K. Kim, G.I. Shulman and A.L. Castle. 2000. Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. J. Clin. Invest. 105: 271–278.
29. Gibson, W.T., I.S. Farooqi, M. Moreau and R.A. Trussell. 2004. Congenital leptin deficiency due to homozygosity for the Delta133G mutation: report of another case and evaluation of response to four years of leptin therapy. J. Clin. Endocrinol. Metab. 89: 4821–4826.
30. Harris, R.B., J. Zhou, S.M. Redmann, G.N. Smagin, S.R. Smith and E. Rodgers E. 1998. A leptin dose-response study in obese (ob/ob) and lean (+/?) mice. Endocrinol. 139: 8–19.
31. Haynes, W.G., D.A. Morgan, S.A. Walsh, A.L. Mark and W.I. Sivitz. 1997. Receptor-mediated regional sympathetic nerve activation by leptin. J. Clin. Invest. 100: 270–278.
32. Heiman, M.L., R.S. Ahima, L.S. Craft, B. Schoner, T.W. Stephens and J.S. Flier. 1997. Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. Endocrinol. 138: 3859–3863.
33. Heymsfield, S.B., A.S. Greenberg, K. Fujioka, R.M. Dixon, R. Kushner and T. Hunt. 1999. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. J. Am. Med. Assoc. 282: 1568–1575.
34. Javor, E.D., E.K. Cochran, C. Musso, J.R. Young, A.M. Depaoli and P. Gorden. 2005. Long-term efficacy of leptin replacement in patients with generalized lipodystrophy. Diabetes. 54: 1994–2002.
35. Javor, E.D., M.G. Ghany, E.K. Cochran and P. Gorden. 2005. Leptin reverses nonalcoholic steatohepatitis in patients with severe lipodystrophy. Hepatol. 41: 753–760.
36. Kelesidis, T., L. Kelesidis, S. Chou and C. S. Mantzoros. 2010. Narrative review: the role of leptin in human physiology: emerging clinical applications. Ann. Intern. Med. 152: 93–100.
37. Kelesidis, T. and C.S. Mantzoros. 2006. The emerging role of leptin in humans. Pediatr.
38. Kim, J.K., O. Gavrilova, Y. Chen, M.L. Reitman and G.I. Shulman. 2000. Mechanism of insulin resistance in A-ZIP/F-1 fatless mice. J. Biol. Chem. 275: 8456–8460.
39. Lee, J.H., J.L. Chan, E. Sourlas and V. Raptopoulos. 2006. Recombinant methionyl human leptin therapy in replacement doses improves insulin resistance and metabolic profile in patients with lipoatrophy and metabolic syndrome induced by the highly active antiretroviral therapy. J. Clin. Endocrinol. Metab. 91: 2605–2611.
40. Licinio, J., C. Mantzoros, A.B. Negrao, G. Cizza, M.L. Wong and P.B. Bongiorno. 1997. Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. Nat. Med. 3: 575–579.
41. Licinio, J., S. Caglayan, M. Ozata, B.O. Yildiz, L. Liang, P. Cohen and M.L. Wong. 2004. Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults. Proc. Natl. Acad. Sci. USA. 101: 4531–4536.
42. Magkos, F., A. Brennan, L. Sweeney and E.S. Kang. 2011. Leptin replacement improves postprandial glycemia and insulin sensitivity in human immunodeficiency virus-infected lipoatrophic men treated with pioglitazone: a pilot study. Metabolism. 60: 1045–1049.
43. Mantzoros, C., J.S. Flier, M.D. Lesem, T.D. Brewerton and D.C. Jimerson. 1997. Cerebrospinal fluid leptin in anorexia nervosa: correlation with nutritional status and potential role in resistance to weight gain. J. Clin. Endocrinol. Metab. 82: 1845–1851.
44. Mantzoros, C.S., J.S. Flier and A.D. Rogol. 1997. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. J. Clin. Endocrinol. Metab. 82: 1066–1070.
45. Mantzoros, C.S. 2010. W(h)ither metreleptin for lipodystrophy and the metabolic syndrome? Endocr. Pract. 16: 162–166.
46. Miller, K.K., M.S. Parulekar, E. Schoenfeld, E. Anderson, J. Hubbard and A. Klibanski. 1998. Decreased leptin levels in normal weight women with hypothalamic amenorrhea: the effects of body composition and nutritional intake. J. Clin. Endocrinol. Metab. 83: 2309–2312.
47. Moon, H.S., J.P. Chamberland, B. Schneider and C.S. Mantzoros. 2011. Leptin and amylin act in an additive manner to activate overlapping signaling pathways in peripheral tissues: in vitro and ex vivo studies in humans. Diabetes Care. 34: 132–138.
48. Munzberg, H. 2008. Differential leptin access into the brain--a hierarchical organization of hypothalamic leptin target sites? Physiol. Behav. 94: 664–669.
49. Myers, M.G., M.A. Cowley and H. Munzberg. 2008. Mechanisms of leptin action and leptin resistance. Annu. Rev. Physiol. 70: 537–556.
50. Nishiyama, A., M. Yagi, H. Awano and M. Matsuo. 2009. Two Japanese infants with congenital generalized lipodystrophy due to BSCL2 mutations. Pediatr. Int. 51: 775–779.
51. Niswender, K.D., G.J. Morton, W.H. Stearns, C.J. Rhodes, M.G. Myers and M.W. Schwart. 2001. Intracellular signalling. Key enzyme in leptin-induced anorexia. J. Nat. 413: 794–795.
52. Oral, E.A., V. Simha, E. Ruiz, A. Andewelt, A. Premkumar and P. Snell. 2002. Leptin-replacement therapy for lipodystrophy. N. Engl. J. Med. 346: 570–578.
53. Ozata, M., I.C. Ozdemir and J. Licinio. 1999. Human leptin deficiency caused by a missense mutation: multiple endocrine defects, decreased sympathetic tone, and immune system dysfunction indicate new targets for leptin action, greater central than peripheral resistance to the effects of leptin, and spontaneous correction of leptin-mediated defects. J. Clin. Endocrinol. Metab. 84: 3686–3695.

54. Park, H.K. and R.S. Ahima. 2015. Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism. 64: 24-34.
55. Paz-Filho, G., M.L. Wong and J. Licinio. 2010. Congenital leptin deficiency: diagnosis and effects of leptin replacement therapy. Arq. Bras. Endocrinol. Metabol. 54: 690–697.
56. Petersen, K.F., E.A. Oral, S. Dufour, D. Befroy, and C. Yu. 2002. Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. J. Clin. Invest. 109: 1345–1350.
57. Pinto, S., A.G. Roseberry, H. Liu, S. Diano, M. Shanabrough and X. Cai. 2008. Rapid rewiring of arcuate nucleus feeding circuits by leptin. J. Sci. 304: 110–115.
58. Robertson, S.A., G.M. Leinninger and M.G. Myers. 2008. Molecular and neural mediators of leptin action. Physiol. Behav. 94: 637–642.
59. Sekhar, R.V., S.G. Patel, K. Rehman and F. Visnegarwala. 2011. Effects of rosiglitazone on abnormal lipid kinetics in HIV-associated dyslipidemic lipodystrophy: a stable isotope study. Metabolism. 60:754–760.
60. Sendlhofer, G., G. Brunner, L. Schaupp, A. Wutte, M. Ellmerer and T. Pieber. 2015. Estimation of human leptin concentration in the subcutaneous adipose and skeletal muscle tissues. Eur. J. Clin. Invest. 45: 445-451.
61. Shimomura, I., R.E. Hammer, S. Ikemoto, M.S. Brown and J.L. Goldstein. 1999. Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. J. Nat. 401: 73–76.
62. Sinha, M.K., J.P. Ohannesian, M.L. Heiman, A. Kriauciunas, T.W. Stephens and S. Magosin. 1996. Nocturnal rise of leptin in lean, obese, and non-insulin-dependent diabetes mellitus subjects. J. Clin. Invest. 97: 1344–1347.
63. Strobel, A., T. Issad, L. Camoin, M. Ozata and A.D. Strosber. 1998. A leptin missense mutation associated with hypogonadism and morbid obesity. Nat. Genet. 18: 213–215.
64. Tsiodras, S. and C.S. Mantzoros. 2006. Leptin and adiponectin in the HIV associated metabolic syndrome: physiologic and therapeutic implications. Am. J. Infect. Dis. 2:141–152.
65. Tsiodras, S., A. Perelas, C. Wanke and C.S. Mantzoros. 2010. A leptin missense mutation associated with hypogonadism and morbid obesity. J. Infect. 61: 101–113.
66. Welt, C.K., J.L. Chan, J. Bullen, P. Smith and A. Karalis. 2004. Recombinant human leptin in women with hypothalamic amenorrhea. N. Engl. J. Med. 351: 987–997.
67. Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold, J.M. Friedman. 1994. Positional cloning of the mouse obese gene and its human homologue. J. Nat. 372: 425–432.

PAK. J. FOOD SCI., 27(1), 2017: 53-60
ISSN: 2226-5899

Evaluation of wheat cassava and soymalt composite flour influence on biscuit quality

Adelakun Oluyemisi Elizabeth[1,2*], and Lawal Monsurat Abiodun[2]

[1]Department of Biological Sciences, Covenant University, Ota, Ogun, State, Nigeria

[2]Department of Food Science and Engineering, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria

*Corresponding Author: Olu_yemisi2000@yahoo.com; oeadelakun@lautech.edu.ng

ABSTRACT

This study evaluated the effect of soy malt addition on properties of cassava-wheat composite flour and its suitability for biscuit production. Various mixes of cassava - wheat - soybean malt flours were analyzed for proximate composition, physicochemical properties, mineral and vitamin. Biscuits were produced from these various flour mixes and the acceptability determined by sensory evaluation. There were increases for crude protein, fiber, ash and fat as soy-malt flour addition increases and ranged from 14.52 - 21.96, 1.52 -1.8, 2.66 - 3.01 and 3.97 - 10.02%, respectively, while the moisture, carbohydrate, sugar and starch content decrease and ranged from 8.12 - 8.73, 59.23 - 54.39, 1.70 - 1.46 and 73.14 – 68.15%, respectively. Physico- chemical properties such as swelling capacity, solubility, bulk density was found to increase and ranged from 237.50 - 257.00%, 227.5 - 265.0% and 0.64 - 0.71 g/cm^3, respectively. The phosphorus, magnesium and potassium content ranges from 7.22 - 11.2, 9.25-12.69 and 1.37-1.24 mg/100g, respectively. The vitamin B_2 and vitamin E content increases with increasing level of cassava and soybean malt flour and range between 0.053-0.068 and 2.44-3.93 mg/100g, respectively. Sensory evaluation indicated that biscuit made from wheat-cassava-soymalt flour mixture with ratio of 70:15:15 compare favourably well with biscuit made from 100% wheat flour in terms of overall acceptable to the panellists. The results suggest that biscuits could be made from the different flour mixes and that soybean malt flour can be used for biscuit enrichment.

Keywords: Wheat, Cassava, Soybean, Biscuit quality, malt,

INTRODUCTION

Consumption of plant protein in the form of soybeans is one of the economical ways of preventing malnutrition because soybean is within the reach of many and possesses relatively high protein content (11TA, 1998). Protein present in soybean are used to replicate the viscoelastic properties of gluten present in wheat dough (Ribotta *et al*., 2004), as such soy flours can be partially substituted for wheat flour at contents up to 30% to produce different variety of baked products (Shogren *et al*., 2003). Although soybean is reportedly high in protein content, complete replacement of wheat flour by 100% of soy flour is however hard to achieve in bakery products because of the resulting beany flavor and compact texture. One of the continuing difficulties to the acceptance of soybean food products is their beany taste, which is caused by the lipoxygenase catalyzed oxidation of unsaturated fatty acid in soybean oil to volatile compounds. Studies have examined the removal of the beany flavor of soybean through germination. This undesirable flavor, as well as the presence of lipoxygenase isozymes that are the disadvantages associated with raw soybean have been reported to be overcome in many countries through germination (Mostafa and Rahma, 1987). Health benefits of soybeans can also be joined in sprouts generated during germination (Kumar *et al.*, 2006). During the process of germination, the chemical compositions of the seed are changed, because the biochemical activity produces essential compounds and energy, for the formation of the seedling. Activation of hydrolytic enzymes occur which decay large molecular substances, such as starch, non-starch polysaccharides and proteins, to small molecular compounds. An increase of simple sugars, peptides and the amino acids of germinated seeds occur due to these processes (Yang *et al*., 2001; Rimsten *et al*. 2003; Saman *et al*., 2008). In addition to the change of the level of nutrients and the biochemical activities that occur during germination, bioactive components are also created (Fernandez-Orozco *et al*., 2008).

Soybean from which malt (controlled germination) can be formed is significant source of protein, meat-like products for vegetarians and for patients with special restraint such as controlled level of fat (Liu, 2000). Since soybean is not imported like wheat and

cheaper in Nigeria, it can serve as a way of improving the nutritive value of biscuit. Also, due to cost, can serve as potential of reducing the cost of biscuit if found suitable when combined with wheat flour. Cassava (*Manihot esculenta* Crantz) is main food crop produced in Nigeria but the chief constraint to cassava application is the quick microbial degradation after harvest. Shelf life of cassava roots is about 24-48 hours after harvest. One way to extend the shelf life of cassava is to make a dry product such as flour. In Africa, cassava flour has a great likely to serve as a substitute to wheat flour (Onyango *et al*., 2011), but flour quality consistency is a major drawback (Hershey *et al*., 2000) as well as its poorer baking properties. Cassava flour lacks gluten, has a very limited amylase activity and also differences in the composition of the starch fraction compared to wheat flour (Aryee *et al*., 2006).

Biscuit, a snack consumed mostly by the children and teenagers can form from wheat – cassava – soybean composite flour. Although cassava is high carbohydrate crop with very low protein content (Onabolu, 2001), the soybean flour can help to supplement this and improve protein content of the biscuit. Since animal protein such as meat and fish are expensive in developing countries like Nigeria, alternative plant sources which are cheaper and within reach need to be sought. The purpose of this study is therefore, to produce and assess biscuit from composite flour of wheat, cassava and soybean malt.

MATERIALS AND METHODS

Procurement of materials

Soybean, granulated sugar, baking powder and margarine were purchased from Sabo market in Ogbomoso, Nigeria. Wheat flour was achieved from eagle flour in Ogbomoso and cassava tubers were gotten from a local farm in Ogbomoso, Nigeria.

Sample preparation

Preparation of malt from soybean

Soybean was cleaned, weighed, soaked in water (soy: water; 1:3) for six hours so as to achieve a 45% moisture level. The water was changed after four hours, sodium benzoate was added to avoid fungal growth during germination, and the grain was re-soaked for another two hours. After two hours, the soaked grain was then drained, loaded onto holed tray lined with muslin cloth and covered with moist muslin. The tray was placed in a seed germinator at 20^{o}C and 95% relative humidity for 72 hours. The germinated grain (Plate 1) was dried from 42% to 8% moisture content. The wasted rootlet was gently being brushed off and the malt was ground in a hammer mill. It was preserved in air tight glass jar and kept at low temperature until use (Mehanna and Martin, 1985).

Preparation of cassava flour

The cassava tubers were harvested fresh, peeled manually, washed and grated. The grated mush was packed into holed sack and pressed using the hydraulic press to drain out water after which it was sun-dried and it was then dry milled into flour (Ashaye *et al*., 2015).

Preparation of composite flour mixes

The various flour blends were made by mixing wheat flour, cassava flour and soybean malt flour together. The six flour samples for the study were: wheat flour 100% (A); cassava flour 100% (B); soy malt flour 100% (C); wheat/cassava/soymalt flour 90/5/5% (D); wheat/cassava/soymalt flour 80/10/10% (E) and wheat/cassava/soymalt flour 70/15/15% (F) respectively.

Biscuit preparation

Biscuit was prepared by mixing all the constituents (Flour, sugar, Egg, margarine, Baking powder) together to form a well founded a dough. The dough was then rolled out, cut into the designed shapes, placed on a lubricated tray and then baked in the oven at the temperature of 240 ^{o}C.

Fig. 1. Germinated soybean grain used for malting process

Proximate composition

The samples were examined for moisture, protein (N*6.25), crude fat, ash, crude fibre and carbohydrate resolute difference according to the method described by AOAC (2005).

Physicochemical properties

Bulk density was resolute by the method reported by Okaka *et al.,* (1991). The swelling capacity was resolute by using the method of (Sathe and Salunkhe 1981) also pH values were determined using the standard methods described in AOAC (2005). The water absorption capacities of sample determined by the method of Sosulski *et al*., (1976)

Mineral composition determination

Selected minerals such as phosphorus, magnesium and potassium were mined from dry ash samples and resolute by atomic spectrophotometer (AOAC, 2005).

Determination of vitamins

Vitamin C (Ascorbic acid) was resolute using a spectrophotometer, according to the method of Klein and Percy (1982). The Vitamin B_2 and Vitamin E content of the flour samples were resolute by phosphomolydate method using alpha – tocopherol as the standard (Jayaprakasha *et al.*, 2003).

Sensory evaluation

Biscuit was baked from each sample, detached from the oven, allowed to cool and then served to ten (10) judges. The panelists were made up of the students and workers of the Department of Food Science and Engineering, LAUTECH. The judges were asked to score the biscuit product for taste, colour, breakability, crispiness, rigidity and general suitability, by using a seven (7) point hedonic scale, where 1 to 7 represent's dislike very much to like very much individually and neither like nor dislike was midpoint.

Statistical analysis

Statistical analysis involved the use of the Statistical Analysis System software package. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple range tests at a level of $P < 0.05$. All data were conveyed as means ± SD (standard deviation)

RESULT AND DISCUSSION

Proximate composition of cassava, wheat and soybean flour mix

The proximate composition of cassava wheat and soybean composite flour shown in Table (1). Results showed protein content of samples increased with increasing quantity of soybean and cassava flour and was found to be within the range of 14.52 – 21.96%. The increase in protein content of the mix was as a result of the replacement with soybean which is a higher source of protein. At present, soybean remains the world's most important source of plant protein (Day, 2013). Moreover, malting of the soybean could has well have increased the protein content and this was in assignation to Wang and Fields (1978) who reported that malting, a old-style processing technology may help to improve the nutritional quality of protein. This could be recognized to a synthesis of enzymatic proteins by germinating seed. Soybean malt flour added at different percentages to the composite flour may have had a significant effect on the protein content. The crude fibre was found to increase with increasing level of soybean and cassava flour, it was found to be within the range of 1.52-1.82%. Fibre has been stated to lowers plasma cholesterol in the body, it also falls the incidence of colon cancer and rise the digestibility of food (Bell *et al*., 1990). The Ash content of the flour mixes increases with increased level in the addition of soybean and cassava flour. It was found to be within the range of 2.66 - 3.01%. This also implies a possibility increase in mineral content. The ash content (2.66-3.1%) of the composite flour detected in this study is alike to the ash content (1.7-3.1%) of malted sorghum-soy composite flour reported by Bolarinwa *et al*., (2015) and (2.9%) of composite flour produced from maize-soy flour as reported by Edema *et al*., (2005).

The fat content was also found to rise with the adding of soybean and cassava flour. It was found to be within the range of 3.97 -10.02%. The increase was as a result of replacement with soy flour, since the flour is not defatted. This may be due to the fact that soybean is a rich source of vegetable oils and at present, the world's most important oilseed, used for the extraction of oil (Day, 2013). The moisture content was found to be within the range of 8.73 - 9.95% with sample A which is pure wheat having the highest moisture content surveyed by sample B which is pure cassava and sample C which is pure soybean flour has the lowest content, but with cassava flour and soybean flour substitution, the moisture content was found to be within the range of 8.12 – 8.73%. The lower moisture would be an advantage, because low moisture content improves stability and extend preservation (Nnam, 2002). The moisture content of all the samples were below the 10% moisture level suggested for safe keeping flour samples (SON, 2007). The carbohydrate and starch

Table 1. Chemical properties of cassava, wheat and soybean based composite flour

Sample (%)	A	B	C	D	E	F
Moisture	9.95^{d}±0.14 9	38^{cd}±0.46	7.28^{a}±0.58	8.12^{b}±0.19	8.43^{b}±0.11	8.73^{bc}±0.17
Ash	1.72^{a} ±0.10	3.71^{d} ±0.06	3.10^{d}±0.21	2.66^{b}±0.13	2.87^{bc}±0.04	3.01^{c} ±0.03
Protein	12.09^{b} ±1.25	2.47^{a} ±0.13	34.63^{f} ±1.66	14.52^{c} ±0.42	17.92^{d} ±0.48	21.96^{e}±0.57
Fat	1.63^{a}±0.09	1.13^{a} ±0.11	15.42^{e} ±1.97	3.97^{b} ±0.16	6.96^{c} ±0.15	10.02^{d}±0.06
Fibre	1.38^{b} ± 0.04	2.33^{e} ±0.1	0.31^{a} ±0.14	1.52^{bc} ±0.06	1.72^{cd} ±0.05	1.82^{d}±0.06
Carbohydrates	73.25^{e}±1.55	80.99^{f} ±0.94	39.27^{a} ±1.23	69.23^{d}±0.53	62.12^{c}±0.30	54.39^{b}±0.54
Sugar	1.95^{d}±0.07	0.47^{a}±0.13	1.60^{bc}±0.08	1.70^{c}±0.02	1.60^{bc} ±0.12	1.46^{b}±0.06
Starch	75.91^{d}±2.33	81.27^{e} ±1.00	47.40^{a} ±2.43	73.14^{cd}±0.46	70.61^{bc}±0.78	68.15^{b}±0.43

Values are means of three determinations (n=3); values with different letter on same row are significant ($p<0.05$). Sample A= 100% Wheat flour; B= 100% cassava flour; C= 100% soy-malt flour; D= 90% wheat flour+5% cassava flour + 5% soy-malt flour; E= 80% wheat flour +10% cassava flour + 10% soy-malt flour; F = 70% wheat flour+15% cassava flour + 15% soy-malt flour

Table 2. Physicochemical Properties cassava, wheat and soybean based composite flour

Sample	A	B	C	D	E	F
Swelling capacity	195.00^{b}	95.50^{a}	350.50^{e}	237.50^{c}	255.00^{cd}	257.00^{d}
Solubility	185.0^{b}	93.00^{a}	345.0^{f}	227.5^{c}	245.0^{d}	265.0^{e}
Bulk Density	0.72^{d}	0.53^{a}	0.65^{bc}	0.64^{b}	0.67^{bc}	0.71^{cd}
Water Absorption	206.5^{c}	147.0^{b}	93.00^{a}	199.5^{c}	190.5^{c}	135.0^{b}
pH	5.05^{b}	6.79^{e}	4.55^{a}	6.61^{d}	6.54^{cd}	6.51^{c}

Values are means of three determinations (n=3); values with different letter on the same row are significant ($p<0.05$). Sample A= 100% Wheat flour; B= 100% cassava flour; C= 100% soy-malt flour; D= 90% wheat flour+5% cassava flour + 5% soy-malt flour; E= 80% wheat flour +10% cassava flour + 10% soy-malt flour; F= 70% wheat flour+15% cassava flour + 15% soy-malt flour

Table 3. Mineral Content of cassava, wheat and soybean based composite flour

Sample	A	B	C	D	E	F
Phosphorous	4.90^{b}	34.00^{f}	0.36^{a}	7.22^{c}	8.99^{d}	11.20^{e}
Magnesium	7.14^{b}	1.30^{a}	19.54^{f}	9.25^{c}	11.09^{d}	12.69^{e}
Potassium	1.72^{c}	0.12^{a}	0.06^{a}	1.37^{b}	1.28^{b}	1.24^{b}

Values are means of three determinations (n=3); values with different letter on the same row are significant ($p<0.05$). Sample A= 100% Wheat flour; B= 100% cassava flour; C= 100% soy-malt flour; D= 90% wheat flour+5% cassava flour + 5% soy-malt flour; E= 80% wheat flour +10% cassava flour + 10% soy-malt flour; F = 70% wheat flour+15% cassava flour + 15% soy-malt flour

Table 4. Vitamin Content of cassava, wheat and soybean based composite flour

Sample (mg/100g)	A	B	C	D	E	F
Vitamin C	13.30^{e}	2.26^{a}	5.40^{b}	11.25^{d}	10.63^{cd}	9.53^{c}
Vitamin B_2	0.032^{a}	0.039^{b}	0.046^{c}	0.053^{d}	0.061^{e}	0.068^{f}
Vitamin E	1.14^{a}	0.17^{a}	9.75^{d}	2.44^{b}	2.99^{bc}	3.93^{c}

Values are means of three determinations (n=3); values with different letter on the same row are significant ($p<0.05$)

Table 5. Taste panel Score of biscuit made cassava, wheat and soybean based composite flour

Sample	A	B	C	D	E	F
Color	6.5^{c}	5.1^{ab}	4.5^{a}	5.00^{ab}	5.60^{b}	5.60^{b}
Taste	5.70^{c}	4.00^{ab}	2.90^{a}	5.00^{bc}	4.80^{b}	5.50^{c}
Crumbliness	5.60^{d}	4.10^{b}	3.30^{a}	4.80^{bc}	4.70^{bc}	5.10^{cd}
Crispness	5.90^{c}	4.50^{b}	2.80^{a}	5.10^{bc}	5.00^{bc}	5.60^{c}
Hardness	6.10^{c}	4.40^{b}	2.50^{a}	5.10^{bc}	5.30^{c}	5.40^{c}

Values are means of three determinations (n=3); values with different letter on the same row are significant ($p<0.05$). Sample A= 100% Wheat flour; B= 100% cassava flour; C= 100% soy-malt flour; D= 90% wheat flour+5% cassava flour + 5% soy-malt flour; E= 80% wheat flour +10% cassava flour + 10% soy-malt flour; F = 70% wheat flour+15% cassava flour + 15% soy-malt flour

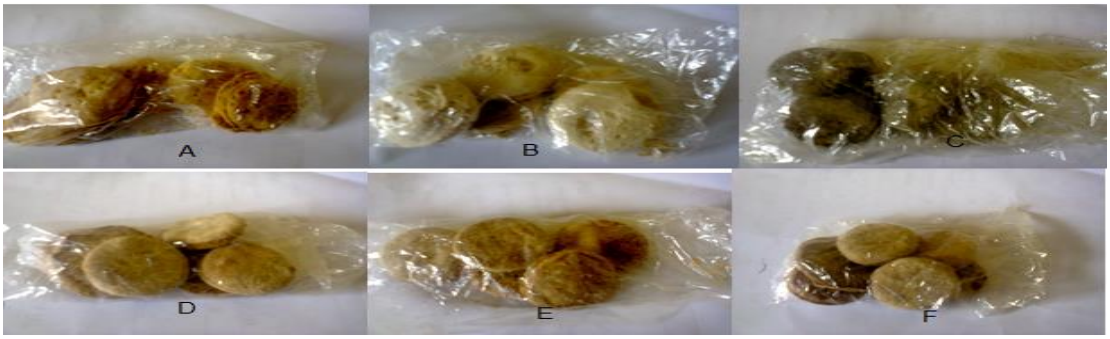

Fig. 2. Biscuits baked from the flour mixes (Sample A= 100% Wheat flour; B= 100% cassava flour; C= 100% soy-malt flour; D= 90% wheat flour+5% cassava flour + 5% soy-malt flour; E= 80% wheat flour +10% cassava flour + 10% soy-malt flour; F = 70% wheat flour+15% cassava flour + 15% soy-malt flour)

contents of the flour mixes losses as the level of soybean flour and cassava flour rises, these were found to be within the range of 54.39 - 69.23% and 68.5 – 73.14% in respectively. It indicated that, the addition of the soybean flour may have contributed to the decreased in carbohydrate and starch contents of the flour mixes. The sugar content decreases with increasing level of soybean flour and cassava flour and was found to be within the range of 1.46 – 1.95%.

Physicochemical properties of composite flour

Table 2 shows the results for swelling ability, solubility, bulk density, water immersion capacity and pH. The swelling ability increases with increasing replacement of soybean malt and cassava flour, and it was found to be within the range of 237.50 - 257.00%. Sample B which is 100% cassava flour had the least and sample C (soybean malt flour) had the highest. Swelling capability is the volume of expansion of molecule in response to water uptake that it possessed until a colloidal suspension is achieved (Houssou and Ayernor, 2002). The increase in the swelling power of the composite flour which was detected as the level of cassava and soybean malt flour replacement increased was as a result of the higher swelling power observed in soybean flour in this work. The solubility of the flour mixes also increases with the addition of flour and cassava soybean and cassava flour C which is pure soybean flour has the maximum solubility (345%), but with the adding of wheat. Sample a flour, it was found to range between 227.5 -265.0%. The Bulk density also increases as a result of cassava and soybean flour substitution and all samples was found to be within the range of 0.53 -0.72 g/cm^3 Cassava flour had the lowest (0.53 g/cm^3) while wheat flour had the highest of 0.72 g/cm^3. The particle size and the density of the food affect the bulk density of food materials. Bulk density is an important factor in food packaging. Low bulk density is desirable in infant feeding (Iwe and Onadipe, 2001) and low bulk density food is desired where packaging is a serious problem (Ikujenlola, 2008).

The water immersion capacity of the flour mixes was found to drop with increasing level of cassava and soybean flour. Sample A that is pure wheat flour has the maximum water absorption capacity of 206.5%. While sample C which is pure soybean flour has the least (93%). Decrease in water absorption ability was

detected as the level of soybean and cassava flour increases and it was found to range from 135.0 - 199.5%. Lower water absorption capacity could be due to absence of gluten in both cassava and soybean when associated with wheat flour. Iwe and Onadipe (2001) reported that capacity of flour to absorb water recovers dough-making potentials. Water absorption capacity of flour is suitable indicator of whether protein can be incorporated with the aqueous food formulations, especially, those involving dough handing (Osungbaro *et al*., 2010). The pH of the flour sample was found to be within the range of 4.55 - 6.79. Sample C which is pure soybean flour has the least pH (4.55) and cassava flour the maximum. The flour mixes pH ranges from 6.51 – 6.61, which shows that the composite flours are not acidic. The flour can therefore be used to produce acceptable products for people suffering from stomach or pectic ulcer. The pH values of the composite flour in this work are similar to the pH values (5.73 to 6.33) reported by Bolarinwa *et al*., (2015) for malted sorghum-soy composite flour.

Mineral content of composition of cassava, wheat and soybean flour mix

The mineral content of cassava-wheat-soybean flour mixes is as shown in Table 3. Minerals are those inorganic elements that have a physiological function in the body (Bender, 2014). Phosphorous content of the flour mixes was observed to increase with increasing level of cassava and soybean flour. It was found to be within the range of 7.22 - 11.20 mg/100g. Sample B that is pure cassava flour has the maximum potassium content (34.00 mg/100g), while sample C that is pure soybean has the least (0.36 mg/100g). The Magnesium content was also found to increase with increasing level of cassava and soybean. It was found to be within the range of 9.25 - 12.69 mg/100g. Sample C which is pure soybean flour has the highest magnesium content, followed by sample A which is pure wheat flour while sample B which is pure cassava flour has the least magnesium content.

The potassium content on the other hand was found to decrease, with increasing level of cassava and soybean flour. It was found to range between 1.37 – 1.245 mg/100g. Sample A which is pure wheat flour has the highest potassium content, followed by sample E which is pure cassava flour while sample C which is pure soybean flour has the least potassium content. Bolarinwa *et al*., (2015) on the hand reported that phosphorous content of the composite flour (malted sorghum-soybean flour) decreases with increased soy flour substitution. This might be because soybean flour had lower phosphorus content as observed in our own study. Higher magnesium level observed in the flour mixes will helps in maintaining normal muscle and nerve functions and keeps the heart rhythm steady. In addition, phosphorus potassium and magnesium that are identified in the samples are all needed in repairing of worn out body cells and making of red blood cells (WHO, 1996).

Vitamin content of cassava, wheat and soybean composite flour

The result for vitamin content of composite flour shown in table (4) reported sample A which is pure wheat flour has the highest vitamin C content, followed by sample C, which is pure soybean flour, while sample B, which is pure cassava flour, has the least vitamin C content, but with substitution with cassava and soybean flour, vitamin C content was found to decrease, and was found to range from 11.25 -9.53 mg/100g.

The Vitamin B_2 content of sample C, which is pure soybean flour is highest followed by sample B, which is pure cassava flour, while sample A has the least vitamin B_2 content, but with substitution and increasing level of cassava and soybean flour, it was found to increase and within the range of 0.053 - 0.068 mg/100g. Also, the vitamin E content of the flour mixes was found to increase and it was within the range of 2.44 - 3.93 mg/100g. Sample C which is pure soybean flour, has the highest vitamin E content, followed by sample A which is pure wheat flour, while sample B which is pure cassava flour, has the least vitamin E content. Vitamins and their biological derivatives are crucial co-factors of many enzymes involved in vital metabolic processes and represent important dietary components essential for health (Langer and Lodge, 2014). Deficiency of riboflavin (vitamin B_2) is linked to cancer, anemia, cardiovascular disease, and various neurological disorders and developmental problems in humans (Powers, 2003). The soybean flour used in this study can be exploited as a basic raw material in composite flour to develop low cost nutritious functional food (biscuit) due to the higher level of vitamins E and B_2 found in it.

Sensory evaluation of biscuits of composite flour

Plate 2 showed the biscuits made from all the flour samples while the results found from organoleptic test are as shown in Table 5. From the table, in terms

of colour, sample E and F are not significantly different from each other. Sample B and D are also not meaningfully altered from each other. Sample A is rated higher while sample C is the least preferred. The taste for samples A, D and F are not significantly different from each other. Sample E and B ranked next while sample C is the least preferred.

For breakability, samples A, D, E and F are not significantly different from each other, sample B ranked next, while sample C is the least preferred. For freshness, all the samples ranked similar, but for sample C, followed by sample B. For hardness sample A is rated higher than all the other samples. Samples D, E and F are not significantly different from each other. The general acceptability shows that sample A which is pure wheat flour is the most acceptable followed by sample F. Samples D and E are not significantly different from each other, followed by sample B while sample C is the least preferred.

CONCLUSION

This study has exposed that biscuit could be made from cassava – wheat – soybean malt flour. The flour mixes were found to be high in protein, vitamin and mineral, which could be of help in avoiding protein-energy malnutrition in children. The high level of protein was because of soybean flour included. Also, cassava flour and soybean malt flour substitution in wheat flour for the assembly of baked product such as biscuit should be encouraged as this may lessen the price of biscuit especially on a large scale and make it more reasonable to the less privileged people in the society.

REFERENCES

1. AOAC 2005. Official method of analysis of the association of official. Analytical Chemist, (18th Edn) Virginia.
2. Aryee, F.N.A., I. Oduro, W.O. Ellis and J.J. Afuakwa. 2006. The physicochemical properties of flour samples from the roots of 31 varieties of cassava. Food Control. 7(11): 916-922.
3. Ashaye, O.A., O.T. Olanipekun and S.O. Ojo. 2015. Chemical and Nutritional Evaluation of Biscuit Processed from Cassava and Pigeon Pea Flour. J Food Process Technol. 6(12): 521.
4. Bell, L.P., K.J. Hectorn, H. Reynolds and D.B. Hunninghake. 1990. Cholesterol lowering effects of soluble-fiber cereals as part of a prudent diet for patients with mild to moderate hypercholesterolemia. Am J Clin Nutri. 52:1020–1026.
5. Bender, A.D. 2015. Introduction to Nutrition and Metabolism. CRC Press, Taylor and Francis group. Pg 307
6. Bolarinwa, I.F., S.A. Olaniyan, L.O. Adebayo and A.A. Ademola. 2015. Malted Sorghum-Soy Composite Flour: Preparation, Chemical and Physico-Chemical Properties. J Food Process Technol. 6(8): 467.
7. Day, L. 2013. Proteins from land plants – Potential resources for human nutrition and food security. Trends Food Sci Technol. 32(1): 25–42
8. Edema, M.O., L.O. Sanni and A.I. Sanni. 2005. Evaluation of maize-soybean flour blends for sour maize bread production in Nigeria. Afr J Biotechnol. 4: 911-918.
9. Fernandez-Orozco, R., J. Frias, H. Zielinski, M.K. Piskula, H. Kozlowska and C. VidalValverde. 2008. Kinetic study of the antioxidant compounds and antioxidant capacity during germination of Vigna radiata cv. emmerald, Glycine max cv. jutro and Glycine max cv. merit. Food Chem. 111(3): 622–630
10. Hershey, C., G. Henry, R. Best and C. Iglesias. 2000. Cassava in Latin America and the Caribbean Resources for Global Development (pp. 1-57).
11. Houssou, P and G. S Ayernor. 2002. Appropriate processing and food functional properties of maize flour. Afr J Sci Technnol. 3(1): 126-131.
12. Ikujenlola, A.V. 2008. Chemical and functional properties of complementary food from malted and unmalted Acha (*Digitaria exilis*), Soybean (*Glycine max*) and Defatted sesame seeds (*Sesamun indicuml*). J Eng Appl Sci. 39(6): 471-475
13. IITA (International Institute of Tropical Agriculture). 1998. Towards sustainable development in Africa. Medium-term Plan 1999–2001. IITA, Ibadan, Nigeria
14. Iwe, M.O and O.O. Onadipe. 2001. Effect of extruded full fat soy flour into sweet potato flour on functional properties of the mixture. J Sust Agri Env. 3(1): 109 – 117.
15. Jayaprakash, G.K., T. Selvi and K.K. Sakariah. 2003. Antimicrobial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. Food Res Int. 36: 117−122
16. Klein, B.P and A.K. Perry. 1982. Ascorbic-acid and vitamin-activity in selected vegetables from different geographical areas of the United-States. J Food Sci. 47: 941–945.
17. Kumar, V., A. Rani., V. Pandey and G.S. Chauhan. 2006. Changes in lipoxygenase isozymes and trypsin inhibitor activity in soybean during germination at different temperatures. Food Chem. 99: 563–568.
18. Langer, S and J.K. Lodge. 2014. Determination of selected water-soluble vitamins using hydrophilic chromatography: A comparison of photodiode array, fluorescence, and coulometric

detection, and validation in a breakfast cereal matrix. J Chromatogr B. 960: 73–81.
19. Liu, K. 2000. Expanding soy bean food utilization. J Food Technol. 54: 46-59.
20. Mehanna, H.T and G.C. Martin. 1985. Effect of seed coat on peach seed germination.
21. Sci Hort. 25:247-254
22. Mostafa, M.M. and E.H Rahma. 1987. Chemical and nutritional changes in soybean during germination. Food Chem. 23: 257-275.
23. Nnam, N.M. 2002. Evaluation of complementary foods based on maize, soybean, pawpaw and mango flour blends. Niger J Nutr Sci. 22 (23): 8 – 18.
24. Okaka, J.C., P.A. Okorie and O.N. Ozo. 1991. Quality evaluation of sun dried yam chips. Trop Sci. 31: 265-275.
25. Onabolu, A.O., O.S. Oluwole, M. Bokanga and H. Roshing. 2001. Ecology variation of intake of cassava food and dietary cyanide load in Nigerian communities. Public Health Nutr 4: 871-876.
26. Onyango, C., C. Mutungi, G. Unbehend and M.G. Lindhauer. 2011. Modification of gluten-free sorghum batter and bread using maize, potato, cassava or rice starch. LWT Food Sci Technol. 44: 681-686.
27. Osungbaro, T.O., D. Jimoh and E. Osundeyi. 2010. Functional and Pasting Properties of Composite Cassava-Sorgum Flour Meals. Agric Biol J North Ame. 1(4): 715-720.
28. Powers, H.J. 2003. Riboflavin (vitamin B-2) and health. Ame J Clin Nutr. 77(6): 1352–1360.
29. Ribotta, P.D., S.F. Ausar, M.H. Morcillo, G.T. Pérez, D.M. Beltramo and A.E. León. 2004. Production of gluten-free bread using soybean flour. J Food Sci Agric. 84: 1969–1974.
30. Rimsten, L., T. Stenberg, R. Andersson, A. Andersson and P. Aman. 2003. Determination of beta-glucan molecular weight using SEC with calcofluor detection in cereal extracts. Cereal Chem. 80(4): 485–490
31. Saman, P., J.A. Vázquez and S.S. Pandiella. 2008. Controlled germination to enhance the functional properties of rice. Process Biochem. 43(12): 1377–1382
32. Sathe, S.K and D.K. Salunkhe. 1981. Functional properties of great northern bean (*Phaseolus vulgaris*) proteins: Emulsion, foaming, viscosity and gelation properties. J Food Sci. 46: 71-75.
33. Shogren, P.L., A.A. Mohamed and C.J. Carriere. 2003. Sensory analysis of whole wheat/soy, flour breads. J Food Sci. 68: 2141–2145
34. SON. 2007. Nigerian Industrial Standard for Pastas. Standard Organization of Nigeria. ICS: 664.68: 1 – 8.
35. Sosulski, F.W., E.S. Humbert, K. Bui and J.D. Jones. 1976. Functional properties of
36. rapeseed flour concentrate and isolate. J Food Sci. 4: 1348–1352.
37. Wang, Y.D and M.L. Fields. 1978. Germination of Corn and Sorghum in the Home to Improve Nutritive Value. J Food Sci. 43(4): 1113-1115.
38. WHO. 1996. Trace elements in human nutrition and health, WHO Technical Report Series, Geneva.
39. Yang, F., T.K. Basu and B. Ooraikul. 2001. Studies on germination conditions and antioxidant contents of wheat grain. Int. J. Food Sci. Nutri. 52(4): 319–330.

PAK. J. FOOD SCI., 27(1), 2017: 61-71
ISSN: 2226-5899

Effect of postharvest dip and storage condition on quality and shelf life of tomato fruits (*Lycopersicon Esculentum* MILL.) in Kura, Nigeria

Dandago Munir Abba[1], Gungula Daniel[2] and Nahunnaro, Hyceinth[3]

[1]Department of Food Science and Technology, Kano University of Science and Technology, Wudil Kano State, Nigeria

[2]Department of Crop Production and Horticulture, Modibbo Adama University of Technology, Yola Adamawa State, Nigeria

[3]Department of Crop Protection, Modibbo Adama University of Technology, Yola Adamawa State, Nigeria

Corresponding Author: dandago223@gmail.com

ABSTRACT

Nigeria is the second largest producer of tomatoes in Africa and 13th largest in the world. It is estimated that total postharvest loss of tomatoes is over 60% which prompted search for simple, cost effective methods to extend shelf life of fresh tomatoes. The present study carried out at Kofar yamma in Kano state investigate the interaction effect of dip and storage condition on quality and shelf life of tomatoes. Green mature tomatoes were harvested and conveyed to site early in morning where sorted, graded and divided into 3 kg lot each. Fruits were given postharvest dips (D_1= dip in tap water, D_2= dip in 200 ppm NaOCl and $NOaCl_2$ for 5 min each, D_3= dip in 200ppm NaOCl and $C_6H_7KO_2$ for 5 and 1 min, respectively) followed by storage condition (S_1= storage at ambient temperature, S_2= storage at refrigerated temperature and S_3= storage at zero energy cool chamber). The analyses on physico-chemical parameters conducted on 1st day and then at a differenc of 3 days for total 18 days. Results indicated dip in 200 ppm NaOCl and 1% $CaCl_2$ for 5 min with refrigeration storage and dip in 200 ppm NaOCl and $C_6H_7KO_2$ for 5 and 1 min, respectively were best treatment combinations that maintained physico-chemical parameters within acceptable limits for 24 days.

Key words: Dip, kura, postharvest, storage, tomato.

INTRODUCTION

The word tomato is a modification of word 'tomati' literally means the swelling fruit. Other names reported by historians are tomatl, tumatle and tomatas (Wikipedia, 2010). Tomato (Lycopersicon esculentum Mill.) is herbaceous plant belonging to family solanaceae., domesticated between Mexico and West coast of South America and following its introduction to Spain in 16th century, widely dispersed throughout African continent (De-Lennoy, 2001). It is one of the popular vegetables worldwide and plays a vital role in human diet (Sibromana et al. 2015). Tomato fruits are consumed whole, in salads, in soups, as juice, ketchup, paste and puree (Adedeji et al., 2005). They are rich in vitamins (particularly A and C), minerals, sugars, essential amino acids, iron, fiber and phosphorus (Ayendiji et al. 2011). Tomato fruits also contain high amount of lycopene, a caretonoid with anti-oxidant properties and beneficial in reducing incidecce diseases like cancer (Basu and Imrhan, 2007) and cardiovascular disorders (Freeman and Reimers, 2010). Nigeria is the second largest producer of tomato fruits in Africa and 13th largest in world (FAOSTAT, 2014). The estimated total postharvest loss of tomatoes in Nigeria is about 60% according to Kutama et al. (2007) which translates to huge economic loss. The huge loss has prompted the search for simple, effective and economical method to control pre-and postharvest diseases and other losses in tomato value chain.

Postharvest technologies like chemical treatments, packaging and storage positively influence the level of postharvest losses and the quality of produce (Srividya et al., 2014). Postharvest treatments such as application of chlorine solutions are known to reduce enzymatic activity and decay by pathogens thereby extending the storage life of the produce (Sood et al., 2011). Zero energy cool chamber (ZECC) is an eco friendly storage system which does not require electric energy. The low inside temperature and high relative humidity of the chamber are based on the principle of passive evaporative cooling mechanism (Islam et al. 2012). Modified atmosphere packaging (MAP) using polymeric films like polyethylene is a simple and inexpensive method to extend the shelf life of fresh fruits and vegetables like tomatoes. In tomato fruits modified atmosphere packaging has been shown to delay ripening and extend the shelf life (Batu and

Thompson, 1995). Extension of the storage life of tomato fruits, regulation of ripening by retarding the metabolic activities coupled with prevention of microbial attack is an important consideration (Sood *et al.*, 2011). An improvement in tomato postharvest handling, packaging and storage is really desirable. The tomato industry can increase the foreign export of many African countries thereby contributing to GDP (Arah, 2015). Therefore this study was aimed at investigating the combined effects of postharvest dip and storage condition on the quality and shelf life of tomato fruits in Kura, Kano State of Nigeria.

MATERIALS AND METHODS

Study plan

The study conducted at Kofar Yamma, Kano State, Nigeria between 2nd March to 27th March, 2014. All analysis were conducted in Laboratories of Department of Food Science and Technology, Kano University of Science and Technology Wudil and Kano laboratory of Abuja Commodity Exchange. Fruits of fairly uniform sizes were carefully harvested at green mature stage early in morning in paper lined plastic crates and they were immediately conveyed to experimental site. The fruits were manually sorted, graded and divided into lots 3 kg and study involve 3x3x3 factorial design in randomized complete block design. Three factor include postharvest dip, packaging and storage having 3 levels. The postharvest dip composition and storage condition is mentioned in table (1). Each treatment consisted of 3 kg sound, unblemished mature green tomato fruits of fairly uniform size dipped in the various dips and subjected to various forms of packaging and stored in the various storage structures. Determinations were conducted on the various physicochemical parameters on day one and thereafter every three days for 24 days.

Fruit firmness

This can be estimated subjectively by finger or thumb pressure but a more precise objective measurement that gives a numerical expression of flesh firmness can be done with a fruit firmness tester. The firmness of tomato fruits was measured with the aid of HP-FFF analog fruit firmness tester (Number 56695 qualitest International Inc., Canada) using 0.25 cm^2 test anvil (specifically for tomato fruits). To test the firmness of the tomato fruits the tester was placed on two different points of the fruit (opposite each other and free of blemishes) with a constant press. The firmness of the fruit was calculated as a quotient of the number directly displayed on the instrument (in kgf). Triplicate determinations were conducted and average calculated.

Percentage weight loss

The percentage weight loss of the stored tomato fruits was determined as a percentage of the initial weight stored. This was done every three days for the period of storage of the tomato fruits.

$$\%\ \text{Weight loss in Tomato} = \frac{\text{Total weight stored} - \text{Final weight}}{\text{Initial weight stored}} \times 100$$

Percentage decay

In the course of storage, rotted fruits were isolated and the percentage rot calculated as a percentage of initial weight of tomato stored.

$$\%\ \text{decay in Tomato} = \frac{\text{Total weight stored} - \text{Weight of rotted fruits}}{\text{Initial weight stored}} \times 100$$

Ascorbic acid content

The ascorbic acid content of tomato was determined by the indophenol method as reported by Onwuka (2005). The fruit was pulped using domestic juice extractor (Master Chef Model MC-J2101). Two grams of the blended pulp was weighed and 100 ml of distilled water added to it in a volumetric flask. The solution was filtered using a filter paper to get a clear solution. Fifty milliliters of unconcentrated juice was then pipetted into 100 ml volumetric flask in triplicate. Twenty five milliliters of 20% Metaphosphoric acid was added as a stablising agent and diluted to 100 ml volume. About 10 ml of the solution was then pipetted into small flask and 2.5 mL of acetone added. The solution was titrated with 2,6-Dichlorophenol indophenol to a faint pink colour which persisted for roughly 15 sec. The amount of ascorbic acid in the tomato fruit was calculated as follows:

$$\text{Vitamin C (mg / 100 g)} = 20 \times V \times c$$

Where V= ml indophenol solution in titration

c= mg vitamin C /mL indophenol

Lycopene content

Fresh tomato fruits were squeezed using potable juice extractor (Master Chef Model MC-J2101) to obtain pure tomato juice. The freshly squeezed sample was drawn into a 100 µl micro pipette and the outside glass bore was wiped clean using tissue paper. The pipette was allowed to stand to dispel air bubbles out of the pipette. The sample was then dispensed into 50 ml separating funnel and closed tightly. Blank samples using 100 µl of water instead of tomato juice was

prepared. Eight milliliters of hexane: ethanol: acetone in ratio 2:1:1 was carefully added immediately and kept out of bright light. After about 10 minutes 1 ml of water was also carefully added and vortex again. The sample was allowed to stand for another 10 minutes to allow the phases to separate and air bubbles disappear. The cuvette of the spectrophotometer was rinsed clean with upper layer from one of the blanks. The liquid was the discarded and another fresh blank was used to zero the spectrophotometer (Jenway Model 752) at 503 nm. The absorption of the upper layers of the sample was then determined using spectrophotometer at 503 nm. Lycopene content was then calculated using the following relationship:

$$\text{Lycopene content } \frac{(A_{503}\times 537\times 8\times 0.55)}{(0.10\times 172)} = (A_{503}\times 137.4)$$

Where: 537 g/mole is molecular weight of lycopene

8 mL is the volume of mixed solvents

0.55 is volume ratio of upper layer to the mixed solvents

0.10 g is the weight of tomato added

172 $mM^{-1}$is the extinction coefficient for lycopene in hexane (Onwuka, 2005)

Statistical analysis

All the data generated in the course of the work were analysed for analysis of variance using GLM procedure of SAS statistical software (SAS Inc. USA Version 9.2 2009) and means that were significantly different were separated using least significant difference method (LSD) at $p\leq 0.001$. The results were interpreted and discussed accordingly.

RESULTS AND DISCUSSION

The results in table 2 presents interaction of postharvest dip and storage on tomato fruit firmness. At 6th day of experiment, fruits firmness decreased generally in all dips with change in storage. As the postharvest dips were changed, a decrease in fruit firmness was observed in storage at ambient room temperature and storage in zero energy cool chamber. Firmness was maintained in storage in zero energy cool chamber after the decrease as the dips were changed to NaOCl with $CaCl_2$ and NaOCl with $C_6H_7KO_2$. In storage at refrigeration fruits firmness were maintained after initial increase as the dips were changed. Fruits dipped in tap water and stored in room at ambient temperature recorded highest firmness of 0.0230 kgf and was therefore the best combination. On 9th day of experiment, a decrease in firmness observed in fruits dipped NaOCl with $CaCl_2$ and NaOCl with $C_6H_7KO_2$, respectively. On other hand, storage in room at ambient temperature recorded an initial increase before a decrease to 0.0214 kgf; while storage in refrigerator recorded a gradual increase in fruit firmness as the dips were changed to dip in NaOCl with $CaCl_2$ and NaOCl with $C_6H_7KO_2$ respectively. Storage in zero energy cool chamber recorded gradual decrease in fruit firmness. Fruits dipped in NaOCl with $CaCl_2$ and stored at ambient room temperature had the highest firmness of 0.0220 kgf and was therefore the best combination. On day 12 of the experiment, fruit firmness decreased in all the dips even though for NaOCl with $C_6H_7KO_2$ firmness was maintained when storage method was changed from refrigeration to zero energy cool chamber. Fruits firmness generally increased in fruits stored at ambient room temperature as the dips were changed. Fruits stored under refrigeration and zero energy cool chamber, followed the same trend of initial increase before decrease as the dips were changed. Fruits dipped in NaOCl with $C_6H_7KO_2$ had the highest firmness of 0.0252 kgf and was therefore the best combination.

On 15th day of experiment, similar trend of initial decrease before an increase was observed in all the postharvest dips as the storage methods were changed. On the other hand, fruits stored under ambient temperature and zero energy cool chamber followed the same trend of decrease, while fruits stored under refrigeration had an increase in firmness before a decrease. The highest firmness value of 0.0232 kgf was recorded in treatment that involved dip in tap water and storage at ambient temperature and this was therefore the best combination. On day 18 of the experiment, a general trend of decrease in fruit firmness before an increase was observed in fruits dipped in tap water and fruits dipped in NaOCl with $CaCl_2$; while an increase was observed in NaOCl with $C_6H_7KO_2$ dipped fruits. An increase in firmness was observed in fruits stored under ambient temperature as the postharvest dips were changed to NaOCl with $CaCl_2$ and NaOCl with $C_6H_7KO_2$ respectively. For fruits stored under refrigeration, fruits firmness was maintained when the dips was changed to NaOCl with $CaCl_2$ and it later decrease when changed to NaOCl with $C_6H_7KO_2$. In fruits stored under zero energy cool chamber, firmness slightly increased as the dip was changed to NaOCl with $CaCl_2$. Fruits dipped in NaOCl with $C_6H_7KO_2$ and stored under ambient temperature gave the highest firmness and was therefore the best combination, while fruits dipped in $C_6H_7KO_2$ and

stored under zero energy cool chamber rotted away and was therefore the worst treatment.

The firmness values ranged from 0.0201 kgf to 0.0252 kgf. The firmness values were lower than 0.0490 kgf reported by Ranatunga et al. (2004). Fruits dipped in Potassium sorbate and stored in room at ambient temperature recorded the highest firmness of 0.0252 kgf. The increased firmness retention here might be due to the effect of the sorbate which according to El-Eryan and El-Metwally (2014) reduces fruit weight loss in tomato fruits; and loss of water through transpiration has also been reported (Kader, 2011) to be responsible for changes in textural quality to fruits. The values for fruit firmness can generally be said to decrease as storage progressed and this could be attributed to increased conversion of hemicelluloses and pectin to simple sugars as reported by Sood *et al.* (2015).

Table 3 presents interaction results of postharvest dip and storage on percentage weight loss of tomato at different storage intervals. The percentage weight loss on day 9 of experiment for all dips followed the same trend of initial decrease before an increase with storage methods were changed to refrigeration and zero energy cool chamber, srespectively. As the postharvest dips were changed, the percentage weight loss in fruits stored at ambient room temperature and those stored in zero energy cool chamber all recorded an increase as the dips was changed to NaOCl with $CaCl_2$ and NaOCl with $C_6H_7KO_2$ respectively. For fruits stored under refrigeration, the percentage weight loss had an initial decrease before an increase. The least percentage weight loss of 0.583 % was recorded in fruits dipped in NaOCl with $CaCl_2$ and stored under refrigeration; and this was therefore the best combination.

On day 12 of the experiment, the percentage weight loss followed the same trend as in day 9 of the experiment. The percentage weight loss in fruits stored at ambient room temperature had an increased trend while it increased and later decreased in fruits stored under refrigeration. In fruits stored in zero energy cool chamber there was a decrease initially before an increase. The best treatment combination was dip in NaOCl with $C_6H_7KO_2$ and storage in refrigerator having the least percentage weight loss of 0.2810%. The percentage weight loss on day 15 also had the same trend as in day 9 and 12. The percentage weight loss in fruits stored in room at ambient room temperature decreased to 6.808% before subsequent increase to 9.710%. Fruits stored under refrigeration as well as under zero energy cool chamber all recorded an increase in the percentage weight loss as the dip was changed to NaOCl with $CaCl_2$ and NaOCl with $C_6H_7KO_2$ respectively. Dip in tap water and storage under refrigerator had the least percentage weight loss of 0.730% and was therefore the best combination.

On day 18 of the experiment, the percentage weight loss in fruits dipped in tap water and those dipped in NaOCl with $CaCl_2$ followed the same trend as in days 9, 12 and 15; while for fruits dipped in NaOCl with $C_6H_7KO_2$ decreased to 0.783% as the storage method was changed to refrigeration. The percentage weight loss decreased in fruits stored at ambient room temperature as well as those stored in zero energy cool chamber as the dips were changed. For fruits stored under refrigeration, there was an increase initially before a decrease as the dips were changed to NaOCl with $CaCl_2$ and NaOCl with $C_6H_7KO_2$ respectively. The least percentage weight loss of 0.783% was recorded in fruits dipped in NaOCl with $C_6H_7KO_2$ and stored under refrigeration and this was therefore the best combination while fruits dipped in NaOCl with $C_6H_7KO_2$ and stored under zero energy cool chamber rotted away and was therefore the worst combination. On day 24 of the experiment, the percentage weight loss in all the dips recorded a decrease as the storage methods were changed. The percentage weight loss in fruits stored at ambient room temperature continually increased as the dips were changed to NaOCl with $CaCl_2$ and NaOCl with $C_6H_7KO_2$ respectively. For fruits stored under refrigeration, the reverse of what happened under ambient conditions was recorded. The best treatment was dip in NaOCl with $C_6H_7KO_2$ and storage under refrigeration because it recorded the least percentage weight loss of 1.086% while dip in NaOCl with $CaCl_2$ and storage in zero energy cool chamber was the worst combination because rotted away.

The percentage loss in weight ranged from 0.281 – 23.770 %. The values were higher than 2.25 – 19.03% reported by Bhatturai and Gautam (2006) but similar to 0.31 – 23.93 % reported by Okolie and Sanni (2012). The highest value of 23.770 % was recorded by treatment dip in potassium sorbate and stored in zero energy cool chamber. The high percentage weight loss could be attributed to the effect of sodium hypochlorite and potassium Sorbate. The results were contrary to the report of Islam and Hatou (2012) and Singh and Yadev (2015) that all reported reduced weight loss in zero energy cool chamber compared to other treatments. Weight loss in stored fruits is usually due to the loss of moisture loss through transpiration which leads to wilting and shriveling as well as due to loss of moisture due to respiration (Znidarcic et al., 2010).

The results in table (4) presented interaction of postharvest dip and storage on percentage rot of tomato fruits for various storage days. It can be observed that on 3rd day of experiment, percentage fruits rot in all three dips drastically before it increased as the storage changed to refrigeration and zero energy cool chamber respectively. As the postharvest dips were changed, the percentage fruit rot in fruits stored under ambient condition and those under zero energy cool chamber increased initially before decreasing as the postharvest dips were changed to NaOCl with $CaCl_2$ and NaOCl with $C_6H_7KO_2$ respectively. For fruits stored under refrigerated condition, the percentage rots initially decrease to 0.008% before increasing to 0.044% as the dips were changed to NaOCl with $CaCl_2$. The least percentage rot of 0.008% was recorded in fruits dipped in NaOCl with $CaCl_2$ and stored under refrigeration and this was therefore the best treatment combination. On day 6 of the experiment, the percentage rot in fruits followed the same trend as in day 3 and dip in NaOCl with $C_6H_7KO_2$ and storage under refrigeration was the best treatment combination.

On day 18 of the experiment the percentage rot for fruits dipped in tap water decreased initially before increasing as the storage methods were changed to refrigeration and zero energy cool chamber. In fruits dipped in NaOCl with $CaCl_2$ and NaOCl with $C_6H_7KO_2$, the percentage fruit rot had a drastic decrease. The best treatment combination was dip in NaOCl with $C_6H_7KO_2$ and storage under refrigeration for having least percentage rot of 0.056% while the worst treatment was dip in NaOCl with $CaCl_2$ and storage in zero energy cool chamber as well as dip in NaOCl with $C_6H_7KO_2$ and storage under zero energy cool chamber having rotted away. Day 21 and 24 followed the same trend as in day 18 of the experiment. The best treatment for day 21 was dip in tap water and storage in refrigerator. The best treatment on day 24 was dip in NaOCl with $C_6H_7KO_2$ and storage under refrigeration. The worst treatment for days 21 and 24 were the same as in day 18 of the experiment.

The interactions for the dip and storage condition were also significant. The percentage rot values ranged from 0 – 58.489 %. The highest percentage rot of 58.48 % and 57.58 % were obtained in fruits dipped in calcium chloride and stored in zero energy cool chamber; and also fruits dipped potassium sorbate for 1 minute and stored in zero energy cool chamber respectively. The results of this study were contrary to the report of Nila et al. (2010) that reported calcium chloride treated fruits stored at ambient temperature recorded a significant reduction in the percentage decay. Arthur *et al.* (2015) also reported a reduction in the percentage decay in Ca^{2+} treated fruits compare to control.

The result of interaction effect of dip and storage condition in fruits ascorbic acids content for different days in storage are presented on Table 5. On day 3 of the experiment the ascorbic acid content in fruits dipped in tap water initially decrease to 21.478 mg/100g before it increased to 33.796 mg/100g. Fruits dipped in NaOCl with $CaCl_2$ recorded an increase in ascorbic acid content while those dipped in NaOCl with $C_6H_7KO_2$ recorded a decrease as the storage methods were changed. As the postharvest dip was changed, the ascorbic acid content in fruits stored in ambient conditions decreased and later increased. The opposite was observed in fruits stored under refrigeration as the dips were changed. Fruits stored in zero energy cool chamber recorded a decreased in the amount of ascorbic acid as the postharvest dip were changed. The best combination was dip in tap water and storage under zero energy cool chamber.

At 9th days, ascorbic acid of fruits dipped in tap water recorded a gradual increase with storage. Fruits dipped in NaOCl with $CaCl_2$ recorded initial decrease before subsequent increase. The trend was reversed in fruits dipped in NaOCl with $C_6H_7KO_2$ as the storage methods were changed. Fruits dipped in NaOCl with $C_6H_7KO_2$ and stored under refrigeration were the best combination. The amount of ascorbic acid in fruits stored under ambient conditions and those stored under zero energy cool chamber increased initially before it later decreased as the dips were changed. The opposite was recorded in fruits stored under refrigeration. The best combination was dip in NaOCl with $C_6H_7KO_2$ and storage under refrigeration. On day 18 of the experiment, the ascorbic acid content in fruits dipped in tap water behaved the same way as in day 9. Fruits dipped in NaOCl with $CaCl_2$ initially increase to 25.506 mg/100g before it decreased to 18.05 mg/100g. Fruits dipped in NaOCl with $C_6H_7KO_2$ recorded a decrease in ascorbic acid as the storage was changed. The amount of ascorbic acid in fruits stored under ambient conditions initially decreased before it later increased as the dips were changed. The opposite of this was observed in fruits stored under refrigeration. Fruits stored under zero energy cool chamber recorded an increase as the dips were changed. Dip in tap water and storage under zero energy cool chamber was the best combination; while dipped in NaOCl with $C_6H_7KO_2$ and stored in zero energy cool chamber was the worst treatment having rotted away.

Table 1. Postharvest dip composition and storage conditions for treatments of tomato

Dip/storage condition	Postharvest dip composition			Storage conditions		
	D_1	D_2	D_3	S_1	S_2	S_3
Description	Fresh harvested tomato fruits dipped in tap water for 5 min	Fresh harvested tomato fruits dipped in 200 ppm sodium hypochlorite for 5 min followed by dipped in 1% w/v calcium chloride for 5 min	fresh harvested tomato fruits dipped in 200 ppm sodium hypochlorite for 5 min and later dipped in 3% potassium sorbate for 1 min.	storage of fresh tomato fruits at ambient room temperature 32 ºC and 29% relative humidity	storage of fresh tomato fruits at refrigerated chamber at 11ºC and 90-95% relative humidity	storage of fresh tomato fruits in "zero energy" cool chamber at 24 ºC and 71% relative humidity

Table 2. Interaction of postharvest dip and storage environment on tomato fruit firmness (kgf) at storage days

Dips	6th day			9th day			12th day			15th day			18th day		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
D_1	0.023	0.0202	0.0208	0.0211	0.0209	0.0211	0.0229	0.0224	0.0213	0.0232	0.0203	0.0216	0.0215	0.0214	0.022
D_2	0.022	0.0204	0.0201	0.022	0.021	0.0208	0.0233	0.0226	0.0221	0.0217	0.0211	0.0214	0.0228	0.0214	0.023
D_3	0.0212	0.0204	0.0201	0.0214	0.0211	0.0207	0.0252	0.0211	0.0211	0.021	0.0208	0.0213	0.0233	0.0208	-
Mean	0.0209			0.0211			0.0223			0.0214			0.0217		
P≤F	0.0145			0.0456			0.0001			0.0001			0.0007		
LSD	0.001			0.0005			0.0007			0.0047			0.0053		

D_1: fruits dipped in tap water for 5 min
D_2: fruits dipped in NaOCl for 5 min and $CaCl_2$ for 5 min
D_3: fruits dipped in NaOCl for 5 min and $C_6H_7KO_2$ for 1 min
S_1: Storage of fruits at ambient room temperature (32ºC and 29% RH)
S_2: Storage of fruits at refrigerated chamber (11ºC and 90-95% RH)
S_3: Storage of fruits in "zero energy" cool chamber (24ºC and 71% RH)
(-): Sample rotted away

Table 3. Interaction of postharvest dip and storage condition on percentage tomato fruit weight loss during storage

Dips	9th day			12th day			15th day			18th day			24th day		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
D_1	2.107	0.633	0.841	2.749	0.493	1.689	9.032	0.73	2.022	11.367	0.906	4.585	5.105	1.698	1.38
D_2	11.499	0.583	2.201	7.943	0.579	1.461	6.808	0.983	3.545	6.305	1.054	3.645	13.443	1.406	-
D_3	14.976	0.691	11.1	9.16	0.281	10.959	9.71	7.576	23.77	6.02	0.783	-	14.593	1.086	-
Mean	4.959			3.722			6.118			3.859			3.705		
P≤F	0.0057			0.0001			0.0003			0.0001			0.0001		
LSD	5.108			2.753			3.878			1.268			0.889		

D_1: fruits dipped in tap water for 5 min; D_2: fruits dipped in NaOCl for 5 min and $CaCl_2$ for 5 min; D_3: fruits dipped in NaOCl for 5 min and $C_6H_7KO_2$ for 1 min
S_1: Storage of fruits at ambient room temperature (32ºC and 29% RH); S_2: Storage of fruits at refrigerated chamber (11ºC and 90-95% RH); S_3: Storage of fruits in "zero energy" cool chamber (24ºC and 71% RH); (-): Sample rotted away.

Table 4. Interaction of postharvest dip and storage condition on percentage tomato fruit rot at various storage interval

Dips	3rd day			6th day			12th day			15th day			18th day			21st day			24th day		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
D_1	2.43	0.06	5.86	6.09	0.09	7.39	10.99	0.57	53.23	26.51	0	22.91	22.37	2.13	18.77	6.79	0	7.275	2.47	1.55	9.06
D_2	9.12	0.01	11.29	13.46	0.06	20.89	23.03	0.06	47.297	20.27	0	58.49	25.26	1.58	-	19.10	1.11	-	15.62	6.44	-
D_3	5.76	0.04	6.45	9.94	0.05	16.98	14.61	0	57.587	12.37	0	35.99	29.023	0.06	-	15.98	2.01	-	3.89	0.63	-
Mean	4.56			8.33			23.37			17.44			10.39			4.43			4.16		
P≤F	0.00			0.01			0.027			0.001			0.000			0.00			0.02		
LSD	1.99			2.97			9.35			22.29						2.134			3.28		

D_1: fruits dipped in tap water for 5 min; D_2: fruits dipped in NaOCl for 5 min and $CaCl_2$ for 5 min; D_3: fruits dipped in NaOCl for 5 min and $C_6H_7KO_2$ for 1 min
S_1: Storage of fruits at ambient room temperature (32ºC and 29% RH); S_2: Storage of fruits at refrigerated chamber (11ºC and 90-95% RH); S_3: Storage of fruits in "zero energy" cool chamber (24ºC and 71% RH); (-): Sample rotted away

Table 5. Interaction of postharvest dip and storage condition on ascorbic acid content (mg/100g) in tomato at various storage intervals

Dips	3th day			9th day			18th day			24th day		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
D_1	22.87	21.478	33.796	14.733	17.586	18.899	20.356	21.636	28.50	24.80	18.505	17.5
D_2	21.70	27.054	32.466	19.677	16.91	22.839	17.867	25.506	18.05	15.483	23.37	-
D_3	27.792	23.04	19.613	18.128	27.814	18.332	20.45	18.758	-	24.067	22.417	-
Mean	25.566			19.425			21.31			21.311		
P≤F	0.0008			0.0095			0.0015			0.0002		
LSD	6.761			5.307			8.195			4.126		

D_1: fruits dipped in tap water for 5 min; D_2: fruits dipped in NaOCl for 5 min and $CaCl_2$ for 5 min; D_3: fruits dipped in NaOCl for 5 min and $C_6H_7KO_2$ for 1 minute
S_1: Storage of fruits at ambient room temperature (32ºC and 29% RH); S_2: Storage of fruits at refrigerated chamber (11ºC and 90-95% RH); S_3: Storage of fruits in "zero energy" cool chamber (24ºC and 71% RH); (-): Rotted

Table 6. Interaction of postharvest dip and storage condition on lycopene Content of tomato at various storage days

Dips	3rd day			6th day			12th day			15th day			21th day		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
D_1	141.741	122.684	167.328	172.851	106.295	176.908	52.344	32.258	87.29	70.969	36.268	78.408	52.147	42.788	125.3
D_2	143.666	89.781	167.591	116.398	111.553	108.568	38.228	43.693	48.874	38.252	25.58	113.638	94.93	32.771	-
D_3	147.777	154.764	154.03	117.083	114.282	133.699	104.232	31.571	77.797	118.016	51.68	57.638	108.08	36.709	-
Mean	142.981			129.406			54.294			58.844			53.328		
P≤F	0.0001			0.027			0.0002			0.0001			0.0005		
LSD	46.700			32.300			20.120			1.268			0.889		

D_1: fruits dipped in tap water for 5 min; D_2: fruits dipped in NaOCl for 5 min and $CaCl_2$ for 5 min; D_3: fruits dipped in NaOCl for 5 min and $C_6H_7KO_2$ for 1 min
S_1: Storage of fruits at ambient room temperature (32ºC and 29% RH); S_2: Storage of fruits at refrigerated chamber (11ºC and 90-95% RH); S_3: Storage of fruits in "zero energy" cool chamber (24ºC and 71% RH); (-): Sample rotted

The ascorbic acid content on day 24 of the experiment for fruits dipped in tap water and those dipped in NaOCl with $C_6H_7KO_2$ decreased as the storage methods were changed. Fruits dipped in NaOCl with $CaCl_2$ recorded an increase as storage method was changed. The ascorbic acid of fruits stored under ambient conditions had the same behavior as in day 21 of the experiment; fruits stored under refrigeration also had the same trend as in day 18 of the experiment. The highest ascorbic acid content was observed in fruits dipped in tap water and stored under temperature ambient conditions. Two treatments dip in NaOCl with $CaCl_2$ and storage in zero energy cool chamber as well as dip in NaOCl with $C_6H_7KO_2$ and storage in zero energy cool chamber rooted away and were therefore the worst treatment combinations. Ascorbic acid values ranged from 14.733 – 33.796 mg/100g. Ascorbic acid values in the present study were higher than the range of 8.33 – 20.07 mg/100g and 17.88 – 21.84 mg/100g reported by Moneruzzaman *et al.* (2009) and Gharezi *et al.* (2012), respectively.

The variation in the amount of Ascorbic acid could be attributed to varietal, soil, and cultural as well as postharvest operation of the fruits. Here also fruits treated with tap water and stored in zero energy cool chamber and fruits treated with Calcium chloride and stored in zero energy cool chamber recorded the highest ascorbic acid content of 33.796 mg/100g and 32.466 mg/100g respectively. Because the two treatments involved ZECC as the storage method, the high ascorbic acid could be attributed to it. Singh and Yadev (2015) reported higher Ascorbic acid retention in tomato fruits stored at zero energy cool chamber compared to other treatments. Table 6 presented the results of interaction of postharvest dip and storage condition on lycopene content of tomato fruits during storage. On day 3 of the experiment, the lycopene content of the fruits dipped in tap water and also in fruits dipped in NaOCl with $CaCl_2$ decreased at initial stage before it increased later as the storage methods were changed. On the other hand fruits dipped in NaOCl with $C_6K_7KO_2$ recorded an increase in the amount of lycopene. There was gradual increase in lycopene content as the postharvest dips were changed in fruits stored under ambient temperature condition. Fruits stored under refrigeration recorded a decrease initially before an increase. Dip in NaOCl with $CaCl_2$ and storage in refrigerator had the least lycopene of 89.781 mg/kg and was therefore the best combination.

The lycopene content in fruits dipped in tap water and those dipped in NaOCl with $C_6K_7KO_2$ on day 6 of the experiment decreased initially before it later increased as the storage methods were changed. Fruits dipped in NaOCl with $CaCl_2$ on the other hand recorded a decrease in the amount of lycopene as the storage methods were changed. The lycopene content in fruits stored under ambient temperature decreased first before it slightly increased later. Fruits stored in zero energy cool chamber had the same trend. On the other hand fruits stored in refrigerator recorded a modest increase in the lycopene content. Dip in tap water and storage under refrigeration had the least lycopene content of 106.295 mg/kg and was therefore the best combination.

The amount of lycopene on day 12 of the experiment for fruits dipped in tap water and also in fruits dipped in NaOCl with $C_6K_7KO_2$ initially decreased before it later increased as the storage methods were changed to refrigeration and zero energy cool chamber respectively. Fruits dipped in NaOCl with $CaCl_2$, on the other hand recorded a fair increase in the lycopene content. The amount of lycopene in fruits stored under ambient temperature and in fruits stored under zero energy cool chamber initially decreased before it later increase. On the other hand, opposite was recorded in fruits stored under refrigeration. The best combination remained dip in NaOCl with $C_6K_7KO_2$ and storage in refrigeration as it had the least lycopene content of 31.571 mg/kg. On day 15 of the experiment, the amount of lycopene in all the three dips initially decreased before it increased later. On day 15 of the experiment, the lycopene content in fruits stored under ambient temperature and those under refrigeration initially decreased before they later increased. The opposite was observed in fruits stored in zero energy cool chamber. Dip in NaOCl with $CaCl_2$ and storage under refrigeration recorded the least lycopene content of 25.580 mg/kg and was therefore the best combination. The amount of lycopene on day 21 of the experiment for fruits dipped in tap water decreased initially before it increased later as the storage methods were changed. The other two postharvest dips all recorded a decrease in the lycopene content as the storage was changed. On day 21 of the experiment, the lycopene content in fruits stored under ambient condition recorded an increase as the postharvest dips were changed to NaOCl with $CaCl_2$ and NaOCl with $C_6K_7KO_2$ respectively. Fruits stored under refrigeration initially decreased before a slight increase as dips were changed. Dip in NaOCl with $CaCl_2$ and storage under ambient temperature had the least lycopene of 32.771 mg/kg as such was the best treatment. The worst treatment was dip NaOCl with $CaCl_2$ and storage in zero energy cool chamber; as well as dip in NaOCl with $C_6K_7KO_2$ and storage in zero energy cool chamber because they all rotted away. Effect of dip and storage on lycopene content

was significant. Lycopene content of tomato fruits ranged from 25.580 mg/kg to 176.908 mg/kg. The ranges were above the 60 – 160 mg/kg and 63 – 155 mg/kg reported by Gharezi et al. (2012) and Markovic et al. (2010) and this could probably be attributed to the different tomato varieties used. The highest amount of lycopene of 176.908 mg/kg was recorded in fruits stored in zero energy cool chamber. Lycopene increased with advancement in maturity and during storage due to loss of chlorophyll and synthesis of color pigments (Hobson and Davis, 1971).

CONCLUSION

Results revealed 200 ppm sodium hypochlorite and 1% calcium chloride dip for 5 min and stored in refrigerated chamber: and 200 ppm sodium hypochlorite dip for 5 min and 3% potassium sorbate for 1 min stored in refrigerated chamber were best treatment combinations to extend shelf life of fresh tomatoes. The two treatments were followed by dip in tap water for 5 min and stored in zero energy cool chamber. That is to say where electricity is a problem, dipping the fruits in clean tap water and storing it in zero energy cool chamber can extend storage life of the fruits**.**

Acknowledgement

The authors wish to acknowledge the assistance of Mal. Sani Abashe and Markus Yari for sample analysis.

Conflict of interest

The authors declare no conflict of interest.

References

1. Adedeji, O, K. Taiwo, C. Akanbi and R. Ajani. 2006. Physico-chemical properties of four Tomato cultivators grown in Nigeria. J. Food Proc. Preser. 30 (1): 79-86.
2. Arah, I. K., H. Amaglo, E.K. Kumah and H. Oferi. 2015. Preharvest and Postharvest Factors affecting the Quality and Shelf life of Harvested Tomatoes: A mini Review. Int. J. Agron. 6: 215. doi 10.1155/2015/478041.
3. Arthur, E., I. Oduro and P. Kumah. 2015. Effect of Maturity Stage and Postharvest Calcium Chloride Treatment on the Quality and Storage life of Tomatoes (*Lycoperson esculectum Mill*). J. Postharvest Technol. 3 (3): 74-81
4. Ayandiji, A.O.R. and O.D. Adeniyi. 2011. Determination of Postharvest losses among Tomato Farmers in Imeko-Afon Local Government Area of Ogun State, Nigeria. Global J. Sci. Front. Res. 11(5): 22-28.
5. Basu, A. and V. Imrhan. 2007. Tomatoes' Versus Lycopene in Oxidative Stress and Carcinogenesis: Conclusions from Clinical Trials. Eur. J. Clin. Nutri. 61 (3): 295-303.
6. Batu, A. and A.K. Thompson. 1995. Effects of Controlled Atmosphere Storage on the Extension of Postharvest Qualities and Storages life of Tomatoes. 1st IFAC/CIGR/EURAGENG/ISHS Workshop, Ostend Belgium June1-2, pp. 311-316.
7. Bhattarai, D. R. and D.M. Gautum. 2006. Effect of Harvesting Methods and Calcium on Postharvest Physiology of Tomato. Nepal Agric. Res. J. 7: 37-41.
8. De-Lennoy, G. 2001. Tomato. In Reamaekers, R. H. (Ed). Crop Production in Tropical Africa. Directorate general for International Co-operation. Brussels, Belgium, pp. 467-475.
9. El-Eryan, E. E. and El-Metwally, M. A. 2014. Enhancing Shelf life of 'Le-Conte' Pear fruits by using Sodium bicarbonate and Potassium sorbate as Postharvest Treatment. Asian J. Crop. Sci. 6 (4): 289-304.
10. FAOSTAT 2014. Global Tomato Production in 2012. FAO, Rome.
11. Freeman. B.B. and K. Reimers. 2010. Tomato Consumption and Health: Emerging Benefits. American J. Lifestyle Med. :1-11.
12. Garcia, J.M., J.M. Ballestros and M.A. Albi. 1995. Effect of Foliar Application of CaCl2 on Tomato Stored at Different Temperatures. J Agri. Food Chem. 43 (1): 9-12.
13. Gharezi, M., N. Joshi and E. Sadeghlan. 2012. Effect of Postharvest Treatment on Stored Cherry Tomatoes. J. Nutri. Food. Sci. 2 (8): 1-10.
14. Hobson, G. and J. Davies. 1971. The Tomato. In the Biochemistry of Fruits and their Products. A.C Hulme Academic press, NY pp. 437-482.
15. Islam, M. P., T. Morimoto and K. Hatou, K. 2012. Storage Behavior of Tomato inside a Zero Energy Cool Chamber. Agric. Engr. Int.: CIGR J. 14 (4): 209-217
16. Kader, A. 2011. Quality and Safety factors: Definition and Evaluation for Fresh Horticultural Crops. In Kader, A. (Ed). Postharvest Technology of Horticultural Crops. 3rd Ed. University of California ANR. PP. 279-285.
17. Kutama A.S., B.S. Aliyu and I. Mohammed, I. 2007. Fungal Pathogens Associated with Tomato Wicker Storage Baskets. Sci. World J. 2 (2): 38-39.
18. Markovic, K., J. Panikota, M. Krpan, D. Bicanic and N. Varcic. 2010. The lycopene Content in Pulp and Peel of Five Fresh Tomato cultivars. Acta Alimetaria. 39 (1): 90-98.
19. Moneruzzaman, K. M., Hossein, A. B. M. S., Sani, W., Saifuddin, M. and Alenazi, M. 2009. Effect of

Harvesting and Storage Conditions on the Postharvest Quality of Tomato (*Lycopersicon esculentum* Mill) CV Roma VF. Aus. J. Crop Sci. 3 (2): 113-121.

20. Onwuka, G.F. 2006. Food Analysis and Instrumentation: theory and practice. Naphthali Prints Lagos, Nigeria. pp. 1-45.
21. Okolie, N.P. and T.E. Sanni . 2012. Effect of postharvest treatments on quality of whole tomatoes. African J. Food Sci. 6 (3): 70-76.
22. Ranatunga, C. L., H.H.E. Jayuweera, S.K.K. Suraweera and T.R. Ariyaratne. 2009. Effects of measurement of non-destructive firmness on Tomato Quality and Comparison with Destructive methods. Proc. Technical Session. 25: 29-35.
23. Sibomana, C. I., Opiyo, A. M. and Aguyoh, J. N. 2015. Influence of soil moisture levels and packaging on post-harvest qualities of tomatoes (Solanum lycopersicum). Afr. J. of Agric. Res. 10 (2): 1392-1400.
24. Singh, Y. and Y.K. Yadav. 2015. Effect of different storage environment on quality characteristics of tomato and kinnow fruits. Agric. Engr. Int.: CIGR Journal. 17 (1): 238-245
25. Sood, M., R.K. Kaul, A. Bhat, A. Singh and I. Singh. 2011. Effects of Harvesting Methods and Postharvest Treatments on Quality of Tomato (*Lycopersicon esculentum*). Annals. Food Sci. Technol. 12(1):58-62.
26. Sood, M., R.K. Kaul and A. Singh. 2011. Effects of Postharvest Treatments on Changes in Sugar and Lycopene Contents of Tomato (*Lycopersicon esculentum*). World J. Agric. Sci. 7(5):613-616.
27. Sood, M., J.O. Bandral and D. Singh. 2015. Effect of Calcium and Modified Conditions on the Post-harvest Quality of Tomato (Lycopersicon esculentum). The Bioscan. 10 (1): 17-22.
28. Srivdya, S., P.S.S. Raddy, K. Umajyothi, V. Sudhavani and R. Reddy. 2014. Effect of Different LDPE Packaging materials on Shelf life and Nutrition Quality of Tomato CV. Lakhshmi under Ambient Conditions. Plant Arch. 14 (2): 1123-1126.
29. Znidarcic, D., D. Ba, M. Oplanic, L. Karic and T. Pozrl. 2010. Influence of Postharvest Temperatures on Physicochemical QQuality of Tomatoes (*Lycopersicon esculentum Mill*). J. Food Agri. Environ. 8 (1): 21-25.