

## EFFICACY STUDY OF ISOFLAVONE CONTENT OF SOYCHEESE IN PREVENTING OSTEOPENIA AND OSTEOPOROSIS

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### ABSTRACT

Osteoporosis is a leading public health cause linked with a considerable socioeconomic burden and mortality as well as morbidity. Osteoporosis is a main cause of fractures in elderly, resulting in pain, early marriages, number of child birth, disability, costly rehabilitation, poor quality of life and premature death. A number of factors can increase the chances of developing osteoporosis some can be changed (modifiable) like low calcium intake, tobacco use, eating disorders, sedentary life style. A large ratio of young Pakistani women was at risk of developing osteoporosis. Drug therapy is not only the source to treat osteoporosis. Some dietary interventions also helps to prevent the adverse effect of the sever diseases. isoflavones are common in prevention of osteoporosis. Soy cheese is rich in isoflavones, calcium, vitamin D and phosphorus. A soy cheese was developed and given for treatment. 15 subjects were selected for the research purpose divided into 4 groups. The research was conducted for 120 days .proximate analysis and polyphenol test was performed to analyze the nutritional profile of soy cheese. The data obtained was subjected to statistical analysis. Significant increasing effect ( $p < 0.000$ ) of soy cheese was observed on the bone concentration of Ca and vitamin D in group t3 and t4 as compare to control group.

**Key Words:** Soy cheese, Osteoporosis, isoflavones, calcium, vitamin D

### Introduction

Osteoporosis is the major health problem in postmenopausal women, that's due to the quick reduction in estrogen value that leads to an increased rate of bone remodeling. According to national institute of health, osteoporosis is defined as a porous disorder that reduces the bone strength and quality of bone to increasing risk of fracture (Kilbanski *et al.*, 2001)

Protein malnutrition is frequent in the elderly and contributes to the development

of osteoporosis. Osteoporotic fractures are a major cause of morbidity in the population. Hip fractures cause acute pain and loss of function, and nearly always lead to hospitalisation. Recovery is slow, and rehabilitation is often incomplete, with many patients permanently institutionalised in nursing homes. Vertebral fractures may cause acute pain and loss of function but may also occur without serious symptoms. Vertebral fractures often recur, however, and the consequent disability increases with the number of fractures. Distal radial fractures

also lead to acute pain and loss of function, but functional recovery is usually good or excellent (Feskenich *et al.*, 2003). The reference range recommended by the different institutes for calculating the T-score is the best way to check the fracture risk in women aged 20-29 years. The diagnostic criteria was same for the men. In men and women risk of hip fracture is a major osteoporotic cause. (Kanis and Gluer, 2000). The continuous increase in population and inadequate supply of protein has inadvertently increased the occurrence of malnutrition in developing countries (Siddhuraju *et al.*, 1996).

The World Health Organization based their definition of osteoporosis on Bone Mineral Density (BMD) that is BMD *t* score of  $\leq -2.5$ . In addition, osteopenia was defined as a BMD *t* score between  $\leq -1.0$  and  $\leq -2.5$  (WHO, 2003).

The factors behind the fracture have been identified. A good factor of fracture is age. The T-score shows the same technique at any one site has slightly significant at any age. The risk of fracture is much higher in the elderly than in the young. Age relevant to risk fracture independently (Kanis JA 2002).

It is predictable that over 10 million Americans over the age of 50 have osteoporosis. Racial background is the major cause for osteoporosis. 132 million have reduced bone mass, is illustrated by osteopenia. In later age they are at high risk of fracture life ([USDH 2004](#)).

Osteoporosis is considered as a 'silent disease' and sometime bone loss might be without any symptoms. Decline of bone tissue and disruption of bone that is the main cause of bone weakness, and minor hit or fall causes a fracture or vertebrae to collapse. It may lead to loss of mobility and independence due to fracture, 25% need long term care ([NAMS 2006](#)).

The prevalence of bone loss is greater in women than in men, and aging is the major orthopedic problems. After the cessation of menses or surgical removal of the ovaries are the main causes of bone loss. The main cause that shows after the menopause is decreased of bone density due to ovarian hormone deficiency that causes an decrease resorption and formation of bones (Carolli *et al.*, 2011). Vitamin D deficiency is a major issue in Pakistan. 8 % hip fracture in Karachi shows osteomalacia. In another study from hazard district; multiparous women were found to be particularly vulnerable to osteomalacia (WHO, 2008).

According to WHO risk for hip, vertebral and forearm fractures has been increased upto 40% same like for coronary heart disease. In the age group of 50 years fracture risk among women is 50 % and 20% in men. Men at the age 75 years have equivalent risk for fracture as women 65 years. In the Philippines hip fracture prevalence over 70 years and above was calculated to be 160 per 10,000 and its cost is 2000 USD per case (Delarosa *et al.*, 2008)

According to National Health and Nutrition Examination Survey III (NHANES III), National Osteoporosis has anticipated that more than 10 million Americans have osteoporosis and an additional 33.6 million have low bone density of the hip (Yu, 2003)

Soybeans are good source of dietary fiber and nutrients magnesium, calcium, vitamin E, and potassium as well as iron. soybeans has a high concentration of estrogen like isoflavones as well as they have high quality protein and healthful fatty acid profile. The basic reason for purposely processing milk into cheese is to preserve a perishable food and to convert it into a stable and storable product. It also expands the variety of food (Liu, 2009).

implemented in different countries (Shamahet *et al.*, 2014). In the study of

women living in the USA, soy foods intake was positively associated with BMD in Japanese but not Chinese women. In the Shanghai women's Healthy study, the 24,403 postmenopausal women followed for 4.5 years had 1770 incident fractures and the soy food intake was assessed at baseline and during follow up using a validated food frequency and soy food intake reduced the major risk factors of osteoporosis, age, socioeconomic status and other dietary factors. The strongest reduction between highest and lowest quintiles of soy food intake for women within 10 years of menopause was 0.52, compared with 0.71 for late postmenopausal women. Similar results were noted for soy isoflavone consumption. (Greendale *et al.* 2002)

The study of 132 women living in the Hong Kong aged between 30 and 40 year were followed for a mean period to determine the effect of soy foods on the maintenance of peak bone mass in the spine. soy food intake remained significant with spine BMD adjusting for factors such as physical activity and energy corrected ca intake. The ability to maintain bone mass after reaching one's BMD peak at 30 year age is generally as being important to fracture risk and soy intake appeared to be associated with less bone loss in this cohort of premenopausal Chinese women. (Ho *et al.*, 2001)

## **MATERIALS AND METHODS**

### **Study Site**

Research has been directed in the Institute of Home Sciences, UAF and further examination had been carried out in post graduate laboratory at Faculty of Food, Nutrition and Home sciences, UAF.

### **Earning of Raw Material**

Soybean seeds were procured from local market of Faisalabad. Cow milk will be purchased from University dairy form.

### **Preparation of Procured Material**

Soybean seeds are used for screening. The seeds were soaked in tape water for 28h and then drain the water and decoated it. After decoating the sample was rinsed twice with tap water then soaked in hot water (100° C) for 20 sec. It will be ground with boil water now sieved with 0.004mm sieve and pasteurized at 68° C for 30 minutes. Soy milk was prepared and then mixed it with cow milk. Lemon will be used as a starter for cheese dehulling.

### **Proximate Analysis of MO Leaf Powder**

The soy cheese was subjected to proximate analysis such as moisture content, crude protein, crude fat, crude fiber and NFE (Nitrogen Free Extract) (AOAC, 2006; AACC 2000).

### **Determination of Minerals**

Minerals i.e. Calcium (Ca) and Phosphorus (P) in pre-treated samples were determined by using Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) following the conditions described in AOAC (1990).

### **Determination of Isoflavones**

Preparation of soybean seeds for high-performance liquid chromatography (HPLC) analysis. The instrumentation for HPLC analysis was used using the method of Hoeck *et al.*, (2008).

### **Efficacy Study**

It was clinical comparative study in which clinical consequences of soy cheese were determined on the bones of osteoporotic test subjects with normal routine diet.

### **Target Population**

The females of 25-55 years of age will be the target population for study. 15 females in this age group and the age group is suffering from deficiency of calcium, phosphate and vitamin D.

### **Sample Collection**

Dexa scan scanning were used for data collection from each individual before

intervention (at 0 day) and at the end of study (at 120day).

### Statistical analysis

Data was analyzed by using SPSS. Paired T test was used to find out relation between dependent and independent variables. Statistical differences between the means of groups were evaluated by one-way analysis of variance (ANOVA). P-values <0.05 were considered significant and P-values <0.01 considered highly significant.(Steel *et al.*,1997).

## RESULTS AND DISCUSSION

### Proximate Analysis

Results of present study revealed that the protein is high in the soy cheese. Soy cheese is reported to have high quality protein which is easily digested and that is influenced by the quality of its amino acids (Foidlet *et al.*, 2001) (Table 1).

### Minerals

Results revealed that soy cheese is providing a significant amount of calcium and phosphorous especially in the diet of lactose-intolerant individuals.

### Isoflavones

Isoflavones are mostly occurring in soybean as a glycosides and high in soy cheese. The three isoflavones genistein, daidzein and glycitein and their respective glycosides account for approximately 50%, 40% and 10%, respectively, of the total isoflavone content of soybeans.

### Efficacy Study of Soy Cheese on Osteoporosis

Significant increasing effect of soy cheese was observed on the bone concentration of Ca and vitamin D osteoporotic test subjects.

**Table 1. Proximate analysis of soy cheese**

<b>Proximate Analysis</b>	
<b>Parameter</b>	<b>Results</b>
Moisture %	69.36%
Crude Protein %	21.29%
Crude fat %	2.6%
Crude fiber %	1.4%
Ash %	3.56%
NFE	17.9%

### Discussion

To achieve the objectives of the study, Soybean seeds were procured from local market of Faisalabad. Cow milk was purchased from University dairy form. Soybean seeds are used for screening. The seeds were soaked in tape water for 28h and then drain the water and decoated it. After decoating the sample will be rinsed twice with tap water then soaked in hot water (100° C) for 20 sec. It was ground with boil water now sieved with 0.004mm sieve and pasteurized at 68° C for 30 minutes. Soy milk was prepared and then mixed it with cow milk. Lemon was used as a starter for cheese dehulling.

**Table 2. Ca and Vitamin D values before and after intervention**

	Ca		Vitamin D	
	Before	After	Before	Aftar
T0	7.2	7.2	22.87	22.87
T1	8.6	9.1	24.23	25.65
T2	8.1	9.2	25.22	28.12
T3	8.7	9.9	23.43	30.72
T4	8.3	10.65	24.34	30.62

It was a cross sectional descriptive study conducted in the Allied Hospital Faisalabad. Females having osteoporosis due to calcium, phosphate and vitamin D deficiency will be selected from the rural areas of Faisalabad. Questionnaire will be developed for the assessment of patients.

The females of 25-55 years of age will be the target population for study. 15 females in this age group and the age group is suffering from deficiency of calcium, phosphate and vitamin D.

Test subjects whom meet the criteria will be divided into 3 groups (A, B, C) by simple random sampling.

Group A was considered as control group with no administration of soy cheese. Group B, group C and group D was experimental groups and given 35 gm of soy cheese respectively.

Significant increasing effect ( $p < 0.000$ ) of soy cheese was observed on the bone concentration of Ca and vitamin D in group t3 and t4 as compare to control group.

## References

AOAC. 2006. *Official methods of analysis of association of official analytical chemists international* In: Horwitz, W 18.  
 Bonjour, J. P. 2011. Calcium and phosphate: a duet of ions playing for bone health. In: Journal of the American College of Nutrition, 30: 438S-448S.  
 Delarosa, M. F., L. Bonifacio and A. Canette. The prevalence assessment of

fragility fractures in the Philippines. In: Journal of Asean Orthopaedic Association 35-38.

Gallardo, C. and E. Perez. 2011. The Clinical Risk Factors of Patients with Osteoporosis at the University of Perpetual Medical Center from 2008 to 2011.

Greendale, G.A., G. FitzGerald, M. H. Huang, B. Sternfeld, E. Gold, T. Seeman. 2002 Dietary soy isoflavones and bone mineral density: results from the study of women's health across the nation. In: Am Journal of Epidemiol 155: 746-754.

Hooper, L., J. J. Ryder, M. S. Kurzer, J. W. Lampe, M. J. Messina, W. R. Phipps and A. Cassidy. 2009. Effects of soy protein and isoflavones on circulating hormone concentrations in pre-and post-menopausal women: a systematic review and meta-analysis. In: Human Reproduction Update 15: 423-440.

Ho, S. C., S. G. Chan, Q. Yi, E. Wong, and P. C. Leung. 2001. Soy intake and the maintenance of peak bone mass in Hong Kong Chinese women. In: Journal of Bone and Mineral Research. 16: 1363-1369.

Kato, K., Y. Takada, H. Matsuyama, Y. Kawasaki, S. Aoe, H. Yano and Y. Toba. 2002. Milk calcium taken with cheese increases bone mineral density and bone strength in growing rats. In: Bioscience, Biotechnology, and Biochemistry 66: 2342-2346.

Klibanski, A., L. Adams-Campbell, T. Bassford, S. N. Blair, S. D. Boden, K. Dickersin and S. R. Johnson. 2001. Osteoporosis prevention, diagnosis, and



therapy. In: Journal of the American Medical Association 285: 785-795.

Lanou, A. J. 2011. Soy foods: Are they useful for optimal bone health? In: Therapeutic Advances in Musculoskeletal Disease 3: 293-300.

Langhammer, A., S. Forsmo and U. Syversen. 2009. Long-term therapy in COPD: any evidence of adverse effect on bone? In: International Journal of Chronic Obstructive Pulmonary Disease 4: 365.

Liu, J., S. C. Ho, Y. X. Su, W. Q. Chen, C. X. Zhang and Y. M. Chen. 2009. Effect of long-term intervention of soy isoflavones on bone mineral density in women: a meta-analysis of randomized controlled trials. In: Bone 44: 948-953

Li-Yu, J., E. Perez, A. Canete, L. Bonifacio, L. Llamado, R. Martinez and C. M. Sison-Pena. 2014. Summary of the Consensus Statements on Osteoporosis Prevention, Diagnosis, and Treatment in the Philippines. In: Journal of the Federation of Endocrine Societies. 27: 156.

Lohana, C. K. and N. Samir. 2016. Risk management of osteoporosis in postmenopausal women; a study of women in a teaching hospital. In: Global Journal of Health Science. 8: 36.

Nazim, M. U., K. Mitra, M. M. Rahman, A. T. M. Abdullah and S. Parveen. 2013. Evaluation of the nutritional quality and microbiological analysis of newly developed soya cheese. In: International Food and Research Journal 20: 3373-3380.

Onuorah, C. E., A. O. Adejare and N. S. Uhiara. 2007. Comparative physico-chemical evaluation of soymilk and soya cake produced by three different methods.

Pinheiro, M. M., R. M. Ciconelli, L. A. Martini and M. B. Ferraz. 2009. Clinical risk factors for osteoporotic fractures in Brazilian women and men. In: Osteoporosis International 20: 399.

Reinwald, S. and C. M. Weaver. 2010. Soy components vs. whole soy: are we betting our bones on a long shot? In: The Journal of Nutrition 140: 2312S-2317S.

Steel, R. G. D., J. H. Torrie & D. A. Dickey. 1997. Principles and Procedures of Statistics In: Biometrical Approach, 3rd Edition. Mcgraw Hill Book Co. Inc., New York, USA.

Taku, K., M. K. Melby, N. Nishi, T. Omori and M. S. Kurzer. 2011. Soy isoflavones for osteoporosis: an evidence-based approach. In: Maturitas 70: 333-338.

World Health Organization. 2003. Prevention and management of osteoporosis. In: World Health Organization.

World Health Organization. 2003. Prevention and management of osteoporosis. In: World Health Organization.

Yamaguchi, M., Z. Ma and T. Fushimi. 2002. Anabolic effect of phosphogenistein and phosphodaidzein on bone components in rat femoral metaphyseal in vitro tissue. In: Journal of Mineral Metabolism 1 55: 71-76.

Zahoor, S. and Z. Ayub. 2011. Prevalence of osteoporosis in postmenopausal women visiting police and services hospital. In: Journal of Postgraduate Medical Institute 24: 234-238

Zhang, X., X. O. H. L. Shu, G. Yang, Q. Li, Y. T. Gao. 2005. Prospective cohort study of soy food consumption and risk of bone fracture among postmenopausal women. In: Arch Intern Med 165: 1890-1895.