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## Tempe Reversed Effects of Ovariectomy on Brain Function in Rats: Effects of Age and Type of Soy Product

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### ABSTRACT

**Background:** Cognitive function when impaired in the elderly is not necessarily indicative of morbidity but it could be a sign of preclinical Alzheimer's Disease (AD) one of the most common forms of dementia in the elderly. Previous observational work in humans suggested that different types of soy products may have different effects on cognitive functions. The aims of this study were to analyze the differences in nutrient content and isoflavones in tempe flour and tofu flour and to analyze the effect of tempe and tofu flour on cognitive function of female rats after ovariectomy.

**Method:** Seventy two (72) white female Sprague Dawley strain rats, aged 12 months were used for this study. Before the intervention 52 rats underwent ovariectomy (OVx) and were grouped into 4 intervention groups: tempe flour (Tp), tofu flour (Tf), estradiol (E2), casein, as a control protein (Cs). The remaining 20 rats were classed as controls without ovariectomy (NO). Cognitive function was measured using a maze test. One-Way ANOVA with Polynomial Contrasts and Post Hoc LSD were used with a p-value <0.05 to indicate significance.

**Results:** The content of nutrients (Vitamin B6, Vitamin B12, folic acid) and isoflavones (genistein) all were higher in tempe flour higher than in tofu flour. After 8 weeks compared to baseline 2 (which was after OVx), the Tp group had showed significantly increased cognitive function (P<0.05), while the Tf group, the eE2 group and the Cs group also all had increased performance, but not significantly so (P>0.05). There was no change in scores in the NO group.

**Conclusions** Intervention by tempe flour can increase cognitive function in female ovariectomized female rats. Further research should focus on other aspects of cognitive function and the content of amyloid plaques and neurotransmitter synthesis in the brain..

**Keywords:** tempe flour, tofu flour, estradiol cognitive function, ovariectomized female rats

## **Background**

Alzheimer's Disease (AD) is a progressive neurodegenerative disease, which is the leading cause of dementia in the elderly and which becomes more common with age (Kandel *et al.* 2002). The prevalence of AD is approximately 1.5% at the age of 65 years, doubling every 4 years and reaching ~ 30% at the age of 80 years. One of the main characteristics of AD is impaired cognitive function impacting on activities of daily life.. Risk factors that may cause cognitive disorders and dementia are varied and include genetic factors, diabetes mellitus, smoking, depression, high blood pressure, high total cholesterol, obesity, cardiovascular disease, lack of physical and social activity, the loss of estrogen and early menopause, and a deficiency of folic acid and vitamin B12 (Seriana 2012; Hogervorst, 2012; Barnes and Yaffe, 2011). Factors that may improve cognitive function may be vitamin B6, vitamin B12, folic acid, isoflavones, hormone replacement therapy (HRT), physical activity / exercise and education (Lee 2010, La Rue 2000; Hogervorst, 2012; Hogervorst, 2011).

HRT is given to increase the levels of estrogen in postmenopausal women. This is done because after menopause, giving estrogen can influence and help regulate functions of the reproductive system, the brain and central nervous system, bones, and the urinary tract. HRT therapy has shown many benefits to the aging process, but the use of hormone therapy comes with an increased risk for some cancers and thrombosis (Kaspers-Kreijkamp et al, 2005) while those for heart disease and dementia may be age-dependent (Hogervorst, 2014).

Various natural and artificial substances have been found to have similar estrogen function (Fang et al., 2001). Artificial substances that are similar to estrogen are called xenoestrogen, while natural ingredients from plants that have similar estrogenic functions are called phytoestrogens. Foods that contain phytoestrogens and are widely consumed in Indonesia are tempe and tofu. Tempe is a fermented soybean product made with molds, whereas tofu is made of soybean curd without fermentation.

Our earlier Indonesian work showed that elderly aged 60 years and over who consumed 75 g of tempe and 150 g of tofu per day had better cognitive function than those who did not (Aryani 2010). A study of community dwelling older adults also showed positive associations between tempe consumption and memory (Hogervorst et al., 2008; 2011). However, those older than 68 years of age, who ate tofu more frequently had lower memory functions and

higher dementia risk than those who reported to eat less (Hogervorst et.al, 2008). Similar associations were reported of tofu on the same memory test in Chinese community dwelling elderly over 68 years of age in Shanghai (Xu, 2014). Earlier research carried out by White et al. (2000) also showed that Japanese American elderly older than 71 years who ate tofu more than twice a week had a higher risk of dementia, brain atrophy and cognitive decline than those consuming less tofu. On the other hand, these associations have not been reported in other Caucasian populations, possibly because they consume on average less tofu (Soni, 2014).

Age may be another factor as we earlier found that optimal levels of genistein for optimal cognitive function, the most potent phytoestrogen, in middle-aged women as compared to older women who showed only negative associations (Hogervorst, 2009). Possibly thus consuming soy products before the age of 68 years can have a positive effect in improving cognitive activity that includes memory and concentration, because isoflavones can have estrogenic effects. Isoflavones are functionally similar to  $17\beta$ -estradiol and the estrogen receptor (ER) is involved in cognitive processes such as learning and memory, as it expresses in the hippocampal formation (HF), amygdala, and cerebral cortex (Anna et al., 2009).

The purpose of this study was to analyze the differences in nutrient content and isoflavones in tempe flour and tofu flour. In addition, the effect of different tempe and tofu flour on cognitive function of female rats with and without ovariectomy was investigated as the rats aged. Our earlier meta-analyses showed that ovariectomy, when done before the natural age of menopause confers risk for accelerated cognitive decline and dementia (Hogervorst, 2014; Soni, 2014)

## **Method**

This research was carried out using an *completely randomized design* (CRD). Spraque Dawley strain female rats (n=72) were obtained from the Faculty of Veterinary Medicine, of Bogor Agricultural University. Rats were kept in cages and given water *ad libitum*. The two weeks after the adaptation period was called *baseline 1*. Rats were then ovariectomized and given a standard feed for 3 months.. Then the rats were given the interventions for 2 months, this included tempe and tofu flour in pellets as well as estradiol as a positive control and casein as a negative control.

In this study, animals were randomly divided into 5 groups : 1) tempe flour after OVx, 2) tofu flour after OVx, 3) estradiol after OVx, 4) casein after Ovx, and 5) casein without Ovx (n=16). One week after the adaptation period, four rats were assessed for cognitive function (baseline 1). 3 months after ovariectomy with standard feed each group of four per treatment group were assessed to establish baseline 2. Before ovariectomy, rats were anesthetized using *ketamine* total of 10% (10-20 mg / kg) and *xylazine* 2% (2 mg / kg). Implementation of surgery was performed by a veterinarian.

Of 52 rats that had undergone ovariectomy, 48 survived and these were allocated to their intervention. Of the non-ovariectomized group (NO) 12 survived.

Investigation of cognitive function was conducted in the Inpatient Installation Animals (IIA), of the Veterinary Hospital at IPB. The intervention was conducted over two months with three observation points; the first in the second week after the intervention, the second in the fifth week and third in the eighth week. At each point of observation, blood was also taken from each rat

Interventions were carried out for 2 months. Feed composition according to the AIN-93M consisted of 14% protein, 5% minerals, 4% fat, 1% vitamins, 5% fiber, 5% water and 66% starch. Total feed consumption was determined by the *fair feeding* of 15 g total feed per rat. The expectation was that each rat consumed the same amount. In the casein group, tempe and tofu flour (how much) was substituted for casein rendering the same amount of protein. In the estradiol group, rats were given ethinylestradiol (synthetic estrogen  $9 \times 10^{-3}$  mg / day / 200g body weight) alongside standard feed using a sonde.

Tests of cognitive function is done by putting a rat in a maze at the start position. Time measurements and observations (problem solving skills) were taken up to five minutes. Assessment of cognitive function category was divided into 4 categories:

Score 4 (Very good): Once put in the maze sniffing activity and walking, climbing walls, and reaching the finish point is less than 5 minutes.

Score 3 (good): Once put in the maze constantly sniffing activity and walking, climbing walls, but does not reach the finish.

Score 2 (fair): Once put in the maze continuously performs activities (smelling) and range from 0.5 minutes to , climbing walls rarely done, does not reach the finish.

Score 1 (poor): Once put in the maze constantly sniffing and walking activities from 0.5 minutes, climbing walls is not done and does, not finish (Mirza 2012).



Figure 1. Maze to measure cognitive function

### **Nutrition and Isoflavone Content Analysis of Tempeh and Tofu Flour**

Nutrients content analysis included: vitamin B6, vitamin B12, folate, and isoflavone content (genistein) using the *High Performance Liquid Chromatography* (HPLC) method. Analysis of proteins was performed using the *Kjedahl* methods.

### **Data Analysis Procedure**

To investigate the difference between treatment groups we used *repeated measures ANOVA* with post hoc LSD polynomial contrast in SPSS 17.0 with a p-value of 0.05 to indicate significance.

## Results

### Substance Content of Isoflavones and Nutrition in Tempe and Tofu flour

The results of the analysis of isoflavones, vitamin B6, folate, and vitamin B12 in tempe and tofu flour using *HPLC* is shown in Table 1. Results of the analysis of proteins using the *Kjedahl* method is also presented in Table 1.

Table 1 Nutrient content and isoflavones of tempe and tofu flour

No	Nutrient	Tempe flour	Tahu flour
1	Isoflavone (genistein) (mg/100 g)	50.56	19.92
2	Folate (mg/100 g)	0.025	-
3	Vitamin B6 (mg/100 g)	0.28	0.19
4	Vitamin B12 (mcg/100 g)	0.97	-
5	Protein (g/100 g)	41.5	47.4

The results showed that tempe flour has twice as much genistein as tofu flour, which also did not contain detectable levels of folate and B12 vitamins and much smaller amounts of B6.

The protein content of tofu flour (47.4%) was slightly but not significantly higher than tempe flour (41.5%).

### Effect of Tempe and Tofu Flour on Cognitive Function

Rats given tempe flour in the second week of the intervention showed they could reach the finish in less than five minutes and were actively engaged until the fifth week (Table 2), but their performance began to decline in the eighth week. Rats given the estradiol intervention showed an improvement up to the fifth week of the intervention but at the eighth week, performance began to fall. In the tofu flour condition, rats showed an improvement until the second intervention week, with a decline in performance from the fifth week, which was significant. Rats given the casein intervention had the lowest cognitive function compared to the other interventions reflecting the ovariectomy effect on memory function. Casein performance was significantly worse at all time points compared to that during tempe which rendered the best performance.

**Table 2 Mean ± SD (95% CI) of cognitive function group of tempe flour, tofu flour, estradiol, casein, non ovariectomy**

Feeding time	Intervention group					P Value
	Tempe flour (Tp)	Tahu flour (Tf)	Estradiol (Es)	Casein (Cs)	Non ovariectomy (NO)	
2 weeks	4.00±0.00 (4.00-4.00)	3.75±0.5 (2.95-4.54)	2.75±0.9 <sup>6</sup> (1.23-4.3)	2.5±1.0 (0.91-4.09)	2.75±1.50 (0.36-5.14)	0.136
5 weeks	4± 0 (4-4)	3.25± 0.5 (2.45-4.05)	3.75± 0.5 (2.95-4.5)	2.75± 0.96 (1.23-4.27)	3.75± 0.5 (2.95-4.54)	0.053
8 weeks	3.75±0.5 (2.95-4.54)	3.5±0.58 (2.58-4.4)	3.0±0.0 (3.0-3.0)	2.75±0.96 (1.23-4.27)	2.75±0.5 (1.95-3.54)	0.100

Using post hoc analyses showed that the tempe flour group had improved cognitive function at all points of observation compared to baseline 2 (P <0.05). The estradiol group only had significantly improved cognitive function at the 5 weeks intervention compared to baseline 2 (P<0.05).

**Table 3. Mean±SD (95%CI) difference in cognitive function between longer treatment with baseline 2**

	Intervention 2 weeks -baseline 2	Intervention 5 weeks -baseline 2	Intervention 8 weeks-baseline 2
Tempe flour	1.25±0.46 <sup>a</sup> (0.28-2.24)	1.25±0.46 <sup>b</sup> (0.26-2.24)	1.0±0.46 <sup>c</sup> (0.01-1.99)
Tofu flour	0.5±0.63 (-0.85-1.85)	0.00±0.63 (-1.35-1.35)	0.25±0.63 (-1.09-1.59)



Estradiol	0.75±0.56 (-0.45-1.95)	1.75±0.56 <sup>d</sup> (0.55-2.95)	1.0±0.56 (-0.19-2.19)
Casein	0.25±0.73 (-1.31-1.81)	0.5±0.73 (-1.06-2.06)	0.5±0.73 (-1.06-2.06)
Non Ovariectomy	0.0±0.84 (-1.78-1.78)	1.0±0.83 (-0.78-2.78)	0.00±0.84 (-1.78-1.78)

Description: The letter that accompanies the figure is a P value = 0.017; b = 0.017; c = 0.048; d = 0.007

## Discussion

The results showed that tempe flour contains twice the levels of genistein (50.56 mg / 100g) of that found in tofu flour (19.92 mg / 100 g). This result is very similar to that of Rahardjo et al. (2010) who showed that the content of isoflavones (genistein) was 55.41 mg / 100 g in tempe flour and 26.68 mg / 100 g in tofu flour. Isoflavones in these studies had a greater value than those of Safrida (2008), where the genistein content in tempe flour was half at 25.06 mg / 100 g. Other studies also found lower genistein levels in tempe (Aryani, 2009) at 38.9 mg / 100 g but similar levels in tofu at 20.8 mg / 100 g. Another study also reported the lower levels of genistein in tempe (Utari, 2011) at 30.8 mg/100 g. Different storage and processing of tempe may have resulted in lower levels found. Here we used a freeze drying method which may have maintained genistein levels better. It may be that the isoflavone content is lower in tofu because when tofu curd is separated in the vinegar water much of the isoflavone remains behind in the fluid. (Pettersson and Kiessling 1984; Taher 2003).

Interestingly in our study, protein levels were similar between tempe and tofu. However, tempe flour has a higher nutrient content than tofu flour because during the fermentation stage the immersion in acidic conditions results in the growth of bacteria for the synthesis of vitamin B2, vitamin B6, vitamin B12, niacin, biotin, folic acid, and pantothenic acid (Hermana and Karmini 1999; Pawiroharsono 2007).

Tempe flour improved cognitive function significantly better than casein and it tended to be superior over estradiol and tofu. This may be the case because soybean flour contains isoflavones, vitamin B6, vitamin B12 and folic acid in higher levels than the tofu flour. Based

on research by Hogervorst et al. (2008) Indonesian elderly who ate tempe had better memory function than those who ate tofu. However, the majority of people who eat both tofu and tempe and it seemed that tempe may have off-set negative effects of tofu in those over 68 years of age. Aryani (2009) found that elderly women who ate tempe (75 g) and tofu (150 g) had better cognitive function (10.94 times greater than not eating either food) than elderly who only ate tofu (7.48 times) or only tempe (1.45 times). In addition, data from Oxford showed that women who had high levels of estrogens but also high levels of folate did not score below the cut-off of dementia while women who had lower folate levels with high estrogens did score below this cut-off (Hogervorst, 2003). It may be that folate protects against (phyto)estrogens by affecting plaque formation. Whereas estrogens and phytoestrogens may exert positive effects on the brain and vascular system in middle-aged women, the effect may be negative in those over the age of 65 years (Brinton-Diaz, 2005)

At the time of the intervention at 5 weeks, of age of rats had the equivalent of humans being over the age of 65, rats given tempeh flour were still cognitively well, but those given tofu flour had decreased performance. These results are consistent with human studies, where administration of HRT at the age of 65 years can increase the risk of dementia (Hogervorst et al., 2008).

Folic acid or folate as found in tempe plays a role in DNA synthesis in producing new cells (Sizer and Whitney 2006). Folic acid functions as a carrier of the methylation cycle. In this cycle, the methyl group of methionine is activated by adenosine triphosphate to form *S-adenosylmethionine* (SAM or AdoMet). SAM is a universal methyl donor involved in methyl transfer reactions of few vital organs, namely the central nervous, being important as a component of the cell membrane synthesis of phosphatidylcholine from phosphatidylethanolamine. Through the transfer of a methyl group, SAM is converted to *S-adenosylhomocysteine* (SAH), which is hydrolyzed to homocysteine. Homocysteine can regenerate methionine for the methylation cycle by acquiring an additional new methyl group of *5-methyltetrahydrofolic acid* in the reaction catalyzed by methionine synthase (Selhub 1999). In liver and kidney, choline can produce labile methyl groups for the regeneration of methionine from homocysteine by *betaine-homocysteine methyltransferase* (BHMT). This alternative pathway is an important way to maintain the methylation capacity during conditions of folate, vitamin B12, and methionine deficiency (Park and Garrow 1999).

Vitamin B12 is necessary to convert folic acid into its active form and function for the normal metabolism of all cells, especially cells of the gastrointestinal, bone marrow system and nerve tissue. Deficiency of vitamin B12 affects cognition, namely the presence of brain atrophy and *white matter* damage in the *spinal cord* and brain. Brain atrophy is a factor that can cause cognitive decline and dementia (Vogiatzoglou et al., 2008). Research of Johnson et al. (2003) showed that low vitamin B12 status is associated with a decrease in the process of remembering (memory recall) and elderly with vitamin B12 deficiency tend to have poor cognitive function. Research on men in Boston by Tucker et al. (2005) found that low plasma concentrations of vitamin B12 can be used to predict cognitive decline.

This result is consistent with previous studies that estrogen affects and helps to regulate the function of the brain and central nervous system that affects the cognitive function (Gibbs 2006; Henderson 2004; Hogervorst 2000a; 2006; Maki 2006). Giving tempeh flour which has twice of isoflavone content than tofu flour will increase estrogenic function. Several studies have shown that isoflavones may improve cognitive function in the elderly (Gleason et al., 2008; Pan et al. 2010) but this may depend on adequate folate and B12 status.

## **CONCLUSIONS AND RECOMMENDATIONS**

### **Conclusion**

The content of nutrients and isoflavones (genistein) in tempe flour was higher than that found in tofu flour and this can increase cognitive function in ovariectomized female rats.

### **Suggestion**

Cognitive function in this study is to measure the creativity of mice to reach the finish within five minutes without any training, so, it further research is needed using other parameters such as measuring memory, plaque, serotonin, dopamine, choline, and neuronal density. Interventions were only carried out up to eight weeks, so there is also a need for further studies with a longer duration of administration to test potential toxicity. Giving tempe flour in different dosages also needs to be done to obtain the optimum dose for maintenance of cognitive function. For the prevention of dementia and other diseases related to estrogen deficiency and further test the age-effect, it is necessary to study both mice at a younger age (premenopausal) and older mice (3 years).

## References

- Seriana van den Berg MD. 2012. Policy brief - Risk factors for dementia Alzheimer's Disease International . [www.alz.co.uk](http://www.alz.co.uk)
- Lee Y. 2012. Systematic review of health behavioral risks and cognitive health in older adults. *International psychogeriatrics*. 22(02):174
- La Rue A, Koehler KM, wayne JS, Chiulli JS, Haaland KY, Garry PJ. 2000. Nutritional status and cognitive functioning in a normally aging sample: a 6-y reassessment. *Am J Clin Nutr*. 65:20-9
- Aryani M.F. 2009. Hubungan Antara Konsumsi Tempe dan Tahu dengan Fungsi Kognitif Lanjut Usia [Desertasi]. Jakarta (ID): Universitas Indonesia.
- White, L.R., Petrovich, H., Ross, G. W., Kamal, M., Hardman, J., Nelson, J., Davis, D., & markesbery, W. 2000. Brain Ageing and Midlife Tofu Consumption-Original Research. *American Journal of Clinical Nutrition*. 19 (2): 242-255
- Eussen SJ, Lisette C. De Groot, Liesbeth W J, Rubia JB. 2006. Effect of oral vitamin B12 with or without folic acid on cognitive function in older people with mild vitamin B12 deficiency: a randomized, placebo-controlled trial. *American Society for Nutrition*. 84(2): 361-370.
- Rahardjo TB, Atik K, Hardinsyah, Hogervorst, Yudarini, Fatmah. 2010. The Effect of Tempeh and Tofu Flours on Delaying Cognitive Function of Elderly Rats. Laporan Penelitian UI Safrida (2008)
- Utari DM. 2011. Efek intervensi tempe terhadap profil lipid, superoksida dismutase, LDL teroksidasi dan malondialdehyde pada wanita menopause [Desertasi]. Bogor (ID): Institut Pertanian Bogor.
- Petterson H and Kiessling KH .1984. Liquid chromatographic determination of the plant estrogens coumestrol and isoflavones in animal feed. *Journal of the Association of Official Analytical Chemists*. 67:503-506
- Taher, A. 2003. Peran Fitoestrogen Kedelai sebagai Antioksidan dalam Penanggulangan Aterosklerosis [Tesis]. Bogor (ID): Institut Pertanian Bogor.
- Wang H-J and Murphy PA. 1994. Isoflavone composition of American and Japanese soybeans in Iowa: Effects of variety, Crop year, and location. *J. Agric. Food Chem*. 42:1674-1677
- Kusbiantoro. 1993. Physicochemical properties and protein characteristics of soybean related to quality of produced tofu Poysa dan Woodrow 2002
- Kiers Jeroen L. 2001. Effects of fermented soya bean on digestion, absorption and diarrhea [Disertation]. Wageningen: Wageningen Universiteit, Netherland.
- Hermana and Karmini M. 1999. The development of tempe technology. Dalam "The complete handbook of tempe". *The American Soybean Association*.
- Pawiroharsno S. 2007. Prospek dan Manfaat Isoflavon untuk Kesehatan. Jakarta: Direktorat Teknologi Bioindustri, Badan Pengkajian dan Penerapan Teknologi. Petterson Christopher. 1994. Screening for Cognitive Impairment in the Elderly. Chapter 75.
- Kurzer M and Xu X. 1997. Dietary phytoestrogens. *Annual Reviews in Nutrition*. 17:353-381
- Nahas Eliana Eguiar Petri and Jorge Nahas-Neto. 2006. The Effects of Soy Isoflavones in Postmenopausal Women: Clinical Review. *Current Drug Therapy*. 1:31-36.
- Wei H, Bowen R, Cai Q, Barnes S, Wang Y. 1995. Antioxidant and antipromotional effect of the soybean genistein. *Proc Soc Exp Biol Med*. 208(1): 124-130.
- Kandel EJ, Schwartz and Jessell T. 2002. Principles of neurogeneration-relevant modifications of brain proteins by dietary soy. *Biofactors*, 12(1-4): 243-250.

- Kiriakidis Serafim, Oliver Ho"gemeier, Susanne Starcke, Frank Dombrowski, Jens Claus Hahne, Michael Pepper, Hem Chandra Jha and Nicolas Wernert. 2005. Novel tempeh (fermented soyabean) isoflavones inhibit in vivo angiogenesis in the chicken chorioallantoic membrane assay. *British Journal of Nutrition*. 93: 317–323
- Kreijkamp-Kaspers S, Kok L, Grobbee DE, de Haan EH, Aleman A, Lampe JW, van der Schouw YT. 2005. Effect of Soy Protein Containing Isoflavones on Cognitive Function, Bone Mineral Density, and Plasma Lipids in Postmenopausal Women: A Randomized, Controlled Trial. *Obstet Gynecol Surv*. 60(1):41-43.
- Sizer F and Whitney E. 2006. Nutrition: concepts and controversies. USA: Thompson Learning, Inc.
- Selhub J, Jacques PF, Boston AG, Wilson PW, Roseberg IH. 1999. The effect of folic acid fortification on plasma folate and total homocysteine concentration. *New England Journal of Medicine*. 340: 1449-1454.
- Park EI and Garrow TA. 1999. Interaction between dietary methionine and methyl donor intake on rat liver betaine-homocysteine methyltransferase gene expression and organization of the human gene. *J Biol Chem*. 274(12): 7816-24.
- Vogiatzoglou A, Refsum H, Johnston C, Smith SM, Bradley KM, de Jager C, Budge MM, Smith AD. 2008. Vitamin B12 status and rate of brain volume loss in community-dwelling elderly. *Neurology*. 71(11):826-32 Johnson *et al.* (2003
- Tucker KL, Hannan MT, Qiao N, Jacques PF, Selhub J, Cupples LA, Kiel DP. 2005. Low plasma vitamin B12 is associated with lower BMD: the Framingham Osteoporosis Study. *J Bone Miner Res*. 20(1):152-8.
- Gibbs. 2006. In: Rasgon NL (ed). The Effects of Estrogen on Brain Function. In N Rasgon (editor) John Hopkins Press. Baltimore, MD.
- Gleason C, Carlsson C, Barnet JH, Meade SA, Satchell KDR, Atwood CS, *et al.* 2009. A preliminary study of the safety, feasibility and cognitive efficacy of soy isoflavone supplements in older men and women. *Age and Ageing*. 38: 86–93
- Pan M, Li Z, Yeung V, Xu RJ. 2010. Dietary supplementation of soy germ phytoestrogens or estradiol improves spatial memory performance and increases gene expression of BDNF, TrkB receptor and synaptic factors in ovariectomized rats. *Nutrition & Metabolism*. 7:75