Journal of Ageing And Family (JoAF) E-ISSN: 2809-0373
Edition 3, No 2, Oktober 2023

The Organoleptic Test For Tempe-Based Drink Formulation For The Prevention Of Cognitive Function In Elderly Women

Atik Kridawati, Laila Ulfa
Graduate Program, Respati University of Indonesia
Faculty of Health Sciences, Respati University of Indonesia
atik@urindo.ac.id

Abstract

Older women are at twice the risk of developing Alzheimer's Disease than men. This happens because when women experience menopause, estrogen in the body will decrease. Estrogen is a hormone that can influence and help regulate body functions including the reproductive system, brain and central nervous system, bones, liver and urinary tract. Hormone replacement therapy (HRT) is intended to increase estrogen levels in postmenopausal women. One of the food ingredients that contain phytoestrogens and is widely consumed in Indonesia is tempe. The aim of the study was to make a tempe-based drink containing isoflavones (genestein, daidzein, glycetin) and nutrients (vitamin B3, vitamin B6, vitamin B12, folate) that are appropriate and sensory acceptable. The content of isoflavones and nutrients was analyzed using High Performance Liquid Chromatography (HPLC). Tempe flour which has been analyzed for its nutritional content and isoflavones, then a tempe drink formulation is made with isoflavones content, namely 15 milli grams (30 grams of tempe flour). To improve the flavor of the drink, add cocoa powder. The design used was a completely randomized design (CRD) with a single treatment, namely the concentration level of tempe flour for formula 1 (original tempe) = 17.5%, formula 2 (original tempe) = 14% and formula 3 (tempe with added chocolate as flavor). = 14 %. Acceptance of tempeh-based drinks was carried out by organoleptic tests in the form of hedogenic (liking) tests on 30 elderly women. In the first phase of the research that for color, taste, aroma and thickness the most preferred was the third formula with the addition of chocolate and 14% thickness. The conclusion is that tempe flour using the freeze dry method contains isoflavones and nutrients (vitamin B3, vitamin B6, vitamin B12 and folic acid) higher than tempe using the oven method but not significantly. Hedogenic test (favorability test) is the highest with a 14% viscosity formula and there is the addition of chocolate

Keywords*: tempe, elderly women, organoleptic test*

INTRODUCTION

Impaired cognitive function in the elderly is not a disease but can be considered as a preclinical condition of Alzheimer's disease (PA), one of the most common types of dementia in the elderly. Some conditions that include cognitive impairment are Age Associated Memory Impairment (AAMI), Mild Cognitive Impairment (MCI), and Vascular Cognitive Impairment (VCI). Most of the MCI (80%) and VCI (50%) patients will develop dementia 5-6 years later[1]. Studies conducted by Pettersen in early detection of dementia stated that the rate of progressive MCI to dementia is between 6-25% per year[1]. For this reason, treatment is needed to slow down cognitive decline in the elderly and there is a need for early detection and management of risk factors so that they can prevent dementia. Dementia sufferers, 40% are over 65 years old with an incidence rate of 187/100,000 people/year. For dementia there is no difference between men and women

while for Alzheimer's Dementia there are more women with a ratio of 1:6. The incidence of Alzheimer's dementia is strongly related to age, 5% of the population aged over 65 years in America and Europe are Alzheimer's sufferers. At the age of 80 years there are 50% of people with Alzheimer's disease [2]. The pathologic features characteristic of Alzheimer's disease are extracellular neuritic plaques with the main components of fibrillar β-amyloid (Aβ) peptide and intraneural neurofibrillary tangles. The main components of neuritic plaques are fibrillar β-amyloid peptides, while the neurofibrillary tangles consist of pairs of tau hyperphosphorylated protein helical filaments. Risk factors that can cause cognitive impairment and dementia are genetic factors, diabetes mellitus, smoking, depression, high blood pressure, high total cholesterol, obesity, cardiovascular disease, lack of physical and social activity, loss of estrogen and menopause due to surgery, deficiency of folic acid and vitamin B12 [3]. Behavioral factors are smoking, drinking alcohol, while factors that can improve cognitive function are vitamin B6, vitamin B12, folate, isoflavones, education, hormone replacement therapy (HRT), and sufficient physical activity/exercise [4,5]. HRT administration is intended to increase levels of the hormone estrogen in postmenopausal women. This is done because estrogen can affect and help regulate body functions which include the reproductive system, brain and central nervous system, bones, liver and urinary tract. HRT therapy has shown benefits in the aging process, but the use of hormone therapy has risks [6]. Various natural and artificial substances have been found to have estrogen-like activity [7]. Artificial substances that act like estrogen are called xenoestrogens, while natural substances from plants that have estrogen-like activity are called phytoestrogens. Those are hormones found in plants, which are contained in soybeans. Natural hormones are chemical compounds found in plants, which have a similar molecular structure (identical) to the molecular structure of hormones in the human body, which are called bio-identical hormones [8]. One plant can contain more than one group of phytoestrogens. Soybean has the most groups containing isoflavones. The effect of isoflavone administration is similar to that of estrogen administration in menopausal women aged 60 with Alzheimer's disease [9]. The effect of phytoestrogens as neuroprotective has been demonstrated in several studies in cells and animals. Phytoestrogens can also reduce Alzheimer's disease and can affect cognitive through interactions with estrogen receptors [10]. One of the food ingredients that contain phytoestrogens and is widely consumed in Indonesia is tempe. Basically giving tempe at the age before 60 years will have a positive effect, increasing cognitive function which

includes memory and concentration due to the isoflavones content which has an estrogenic effect. Isoflavones are the predominant phytoestrogens, structurally are estrogen-like substances and functionally similar to 17β-estradiol. Estrogen hormone is distributed throughout the body including the central nervous system or central nervous system (CNS). Estrogen receptors (ER) are involved in cognitive processes such as learning and memory, the formation of the hippocampus (HF), amygdala, and cerebral cortex [11]. The results of research on tempe showed an effect of increasing cognitive function and decreasing serum amyloid beta in ovariectomized female rats and old age [12,13,14,15]. In epidemiological studies conducted in Yogyakarta, Sumedang and Yogyakarta also showed an increase in cognitive function in elderly women [16]. Elderly people who consume tempe have better cognitive function due to the possibility of the presence of vitamin B12 and folic acid in tempe [17]. Research [18] using 100 mg of total isoflavones given daily to subjects for 10 weeks, showed an increase in cognitive function in female subjects. According to [19], 100 mg of total isoflavones is equivalent to 125 g of tempe (4-5 medium pieces) and 200 g of tofu (4 pieces). Dietary soy isoflavones affect the structural aspects of the brain, learning processes, memory and anxiety along the metabolism of androgen enzymes in the brain in the frontal lobe. Isoflavones have a positive effect on cognitive performance and mood. Soy contains three types of isoflavones, namely daizein, genestein and glicetein. In tempe, besides the three types of isoflavones, there is also antioxidant factor II (6,7,4 trihydroxy isoflavones) which has the strongest antioxidant properties compared to isoflavones in soybeans [20,21] and contains essential amino acids [22]. The aim of this research was to make tempe-based drinks with appropriate and sensory acceptance of isoflavones and nutrients (vitamin B3, vitamin B6, vitamin B12, folic acid). Raising the potential of tempe drink as a functional drink containing isoflavones, vitamin B6, vitamin B12 and folate. The results of this study are very useful for the development of Community Nutrition science, especially clinical nutrition and dietetics and the field of geriatric nutrition from the aspect of healthy and nutritious food (healthy eating) for elderly women.

LITERATURE REVIEW AND HYPOTHESIS DEVELOPMENT

Menopause in women can cause, among others, impaired cognitive function. Impaired cognitive function if not treated quickly will be the cause of Alzheimer's disease and can develop into dementia. The cause of decreased cognitive function is partly due to the cumulative risk of extrinsic factors, such as food. Research shows the role of dietary isoflavones associated with improving

cognitive function. The main source of isoflavones is soybeans and their processed products. Indonesian people are familiar with and consume isoflavones in soy foodstuffs and their processed products, including tempe, tofu, oncom and tauco. The average consumption of soybeans and their processed products is 49.1 g/capita/day [23] or equivalent to 22.18 mg of isoflavones (Kridawati, 2016). This amount is lower than the Japanese consumption of soybeans (59 g/day). Several Asian countries (Japan, China, Taiwan and Korea) reported consuming isoflavones as much as 20-150 mg/day. Tempe is a traditional Indonesian food which is currently a popular food in the world [24]. AFIC recommendation (2000) for the intake of phytoestrogens that have biological effects is 30-50 mg/day. In a state where the body does not have estrogen, even though it is weak, phytoestrogens can have an estrogenic effect. Ferri (2005) [25] reported the prevalence of dementia for Indonesia, Sri Lanka and Thailand as much as 2.7% higher than South Asia and India (1.9%) but still lower than China and the Developing Western Pacific (4.0%) and Western European countries (5.4%) and North America (6.4%). Although the consumption of soybeans and their processed products in Indonesia is lower than other Asian countries, the prevalence of dementia is lower than in China. This incident may be due to Indonesia consuming tempeh which besides containing high isoflavones also contains SOD and vitamin B12 which are beneficial for cognitive function. Phytoestrogens are a group of biologically active plant substances with the same chemical structure as estadiol, endogenous estrogen [26]. This structure has the same ability to bind to estrogen receptors on various cells [27] and influences estrogenic and antiestrogenic effects. The three main groups of phytoestrogens are isoflavones, lignans and comestans. The main bioactive isoflavones are genestein and daidzein, which are derived into biochanin A precursors and formonetin respectively[26]. Genestein can actively generate estrogen and can inhibit estrogen or antagonism depending on the tissue and the availability of endogenous estrogen components [27] due to the presence of an important phenolic ring for estrogen receptors (ER). Genestein can increase overall uterine growth and ovariectomy in rats. The effect of phytoestrogens on cognition at different ages has the same effect as estrogen [29,30]. Data from the Honolulu Asia Aging Study (HAAS), midlife who consumes a lot of tofu is associated with indicators of cognitive impairment and brain atrophy later in life [31]. In research in Indonesia, high consumption of tofu is associated with memory impairment, but consumption of tempe is associated with better memory, especially in the elderly over 68 years [16]. Preliminary studies on rats aged 1 (one) year that were ovariectomized showed that rats that consumed tempeh flour had

better cognitive function than rats that consumed tofu flour [13]. Likewise with the content of beta amyloid in serum, rats that were given tempeh flour had lower serum amyloid beta than mice that were given tofu flour. This shows that tempe flour is better for preventing Alzheimer's disease. So the authors are interested in further research on elderly women. This is the first year of research, a tempe-based drink formula was produced which was sensory acceptable to prevent cognitive decline.

RESEARCH METHOD

This research was to make a drink formula based on tempe flour. The material used in the manufacture of tempe flour is fresh tempe from the KOPTI tempe factory in Bogor. The process of making tempe in this study used 50 kg of Americana variety soybeans by following the following steps: boiling was carried out for 4 to 5 hours, skin separation, washing, fermentation using yeast mixed with cassava in a ratio of one to ten. Giving yeast 50 grams per 50 kg of soybeans. The yeast used was Rhyzopus oryzae inoculum produced by PT Aneka Fermentasi Industri (AFI) Bandung (BPOM RI MD 262628001051). Tempe is then packaged and stored for 36 hours. For 50 kg of soybeans after the fermentation process will produce ± 75 kg of tempe. The process of making tempe flour is as follows: tempe slicing (1x2x0.5 cm3), milling, drying in the oven method for 2 days at 400C. This analysis includes the nutritional content of tempeh flour (vitamin B6, vitamin B12, folic acid, protein) and isoflavones (genestein, daizein, glycetein). Analysis of protein, vitamin B6, vitamin B12 and folic acid was carried out at the Bogor Post-Harvest Center, Ministry of Agriculture. Isoflavone analysis was carried out at the Nutrition Laboratory, State University of Malang. Protein analysis using the Kjedahl method. Analysis of isoflavones (genestein, daizein, glycetein), vitamin B6, vitamin B12, and folic acid using the High Performance Liquid Chromatography (HPLC) method.

Analysis of Nutritional Content and Isoflavones

This analysis includes the nutritional content of tempe flour (vitamin B6, vitamin B12, folic acid, protein) and isoflavones. Analysis of protein and vitamin B6 was carried out at the Bogor Agro Industry Center, Ministry of Agriculture. Analysis of isoflavones, vitamin B12 and folic acid at the Bogor Post-Harvest Center, Ministry of Agriculture. Protein analysis used the Kjedahl method and analysis of isoflavones (genestein), vitamin B6, vitamin B12, and folic acid used the High Performance Liquid Chromatography (HPLC) method.

Protein Analysis [32]

Destruction process: carried out by weighing ±1 g of the material that has been mashed, put it in the Kjeldahl flask. Then 7.5 g of

potassium sulfate and 0.35 g of mercury (II) oxide and 15 ml of concentrated sulfuric acid were added. Then heat all the ingredients in the Kjeldahl flask in the fume hood until it stops smoking and continue heating until it boils and the liquid becomes clear. added heating for about 30 minutes, turned off the heating and left to cool. Next, 100 ml of aquadest was added in a Kjeldahl flask cooled in ice water and a few Zn plates, added 15 ml of 4% potassium sulfate solution (in water) and finally slowly added 50% sodium hydroxide solution as much as 50 ml which had been cooled in the refrigerator.

Distillation Process:

The Kjeldahl flask is installed in the distillation apparatus. Then the Kjeldahl flask is heated slowly until the two layers of liquid are mixed, then heated rapidly until it boils. The distillate is collected in an Erlenmeyer which has been filled with 50 ml of 0.1 N hydrochloric acid standard and 5 drops of 0.1% b/v methyl red indicator (in 95% ethanol), the tip of the distillator glass tube is ensured to enter into the 0.1 N hydrochloric acid solution. The distillation process is complete when the distillate contained is approximately 75 ml. Titration Process: The remaining 0.1 N hydrochloric acid solution which does not react with the distillate is titrated with 0.1 N sodium hydroxide standard solution. The end point of the titration is reached when the color of the solution changes from red to yellow. Perform a blank titration. The formula for determining crude protein content: Crude Protein (%) = (y-z) x NaOH titar x 0.014 x 6.25(gram) x 100% Sample Weight (gram) Analysis of Vitamin B6 (Amidzic et al. 2005) Standard Solution. The USP standard is weighed which is equivalent to 800 µg of vitamin B6 in 100 ml. Then add water up to 100 ml until completely mixed. This mixture is made in the dark and stored for one week. After one week, 10 ml of the solution was taken, put in a 100 ml measuring cup and mixed with water. This standard solution contains 0.8 µg/ml of vitamin B6. Solution for samples. Samples were weighed 2 g in 125 ml Erlenmeyer flask. Then add 25 ml of water and shake for one minute. Then filtered with 0.45 µ milliphore and injected into HPLC.

Folic Acid Analysis [33]

Preparation of Standard Folic Acid: 0.005 g of folic acid is weighed and put into an Erlemeyer flask, then added with distilled water up to 50 ml and given 15 drops of 0.04 N NaOH to neutralize the pH. Then the solution was taken 5 ml and diluted to 50 ml. The dilution results were taken as much as 0.1 ml, 0.3 ml, 0.5 ml, 1 ml, 3 ml and 5 ml. Each supernatant was taken 0.5 ml for HPLC. Extraction: 1 g of tempe flour and tofu flour each, each put in three test tubes. Furthermore, 4 ml of acetonitrile CH3CN was added, then shaken until well mixed. The solution was added with 3 ml of KOH, 2 ml of

Phosphate Buffer. The first test tube for tempe flour and tofu flour was added with 0.5 ml (10 ppm) standard folic acid. Another test tube was added with 0.5 ml TCA and buffer to a volume of 10 ml. The solution is filtered. The supernatant was taken 0.5 ml for HPLC. Isoflavone Analysis (Mantovani et al. 2009) 2 g of sample was dried at 400C for 5 hours. Samples were extracted with MeOH absolute 2 times, each 100 ml filtered. Then it was left in the room at 00C for 1 hour to coagulate the fat. Then the fat was separated by centrifuging at 2000 rpm for 20 minutes. The supernatant was taken and evaporated at 70 oC. The residue was dissolved in 10 ml of 60% MeOH and centrifuged at 4000 rpm for 20 minutes. Dry on the rotary evaporator at 700C. Then it was dissolved with 10 ml HPLC grade MeOH and passed to the Shepak C18 cartridge. Filtered with 0.2 micron milliphore and injected into HPLC.

Analysis of Vitamin B12[33]

Standard Solution. The USP standard is weighed which is equivalent to 800 µg of vitamin B6 in 100 ml. Then add water up to 100 ml until completely mixed. This mixture is made in the dark and stored for one week. After one week, 10 ml of the solution was taken, put in a 100 ml measuring cup and mixed with water. This standard solution contains 0.8 µg/ml of vitamin B12. Solution for samples. Samples were weighed 2 g in 125 ml Erlenmeyer flask. Then add 25 ml of water and shake for one minute. Then filtered with 0.45 µ milliphore and injected into HPLC. Tempe flour, which has been analyzed for its nutritional content and isoflavones, is then made into a tempe drink formulation with isoflavones content of 15 -144 milligrams/day (Kridawati et. al. 2013, 2015; Whitten and Patisau 2001). The lowest isoflavone content in this study was used, namely 15 milli grams (30 grams of tempeh flour) because the respondents were elderly. To improve the flavor of the drink, add cocoa powder.

The design used was a completely randomized design (CRD) with a single treatment, namely the concentration level of tempe flour for formula 1 (original tempe) = 17.5%, formula 2 (original tempe) = 14% and formula 3 (original tempeh with the addition of 1% chocolate as flavor) = 14%. Acceptance of tempe-based drinks was carried out by organoleptic tests in the form of hedonic (liking) tests on 30 elderly women. In the hedonic test (liking) the observed parameters are color, aroma, taste, thickness. The range of the scale used is a scale of 1 = really really don't like it to 9 = really like it very much. Data from the hedonic test were analyzed using the Fiedman test, then a multiple comparison test was performed.

RESULTS AND DISCUSSION

Isoflavones and Nutrients in Tempe Flour

The isoflavone content analyzed in tempe flour was genistein, daidzein and glycetin

using the HPLC method. The nutrients analyzed included vitamin B3, vitamin B6, vitamin B9 (folic acid) and vitamin B12 using the HPLC method. Protein analysis with Kjedahl.

Table 1. Nutrient Content of Oven and Freezedry Tempe Flour

No	nutrient Content	Oven Tempe Flour	Freezedry Tempe Flour
1	Genistein (mg/100 g)	36,27	41,21
2	Daidzein (mg/100 g)	27,13	29,80
3	Glycetin (mg/100 g)	3,63	6,59
4	Vitamin B3 (mg/100 g)	97,4	125,52
5	Vitamin B6 (mg/100 g)	0,423	0,548
6	Folic Acid (mcg/100 g)	27,59	47,15
7	Vitamin B12 (mcg/100 g)	0,363	0,296
8	Protein (g/ 100 g)	41,5	47,4

From table 1 it can be seen that the isoflavones in tempe flour using the freeze dry method are higher than using the oven although statistically they are not significantly different (p>0.05). The content of vitamin B3, vitamin B6, and folic acid in freeze drying tempe flour is also higher than an oven tempe flour. But for vitamin B12 obtained freeze drying tempe flour is lower than oven tempe flour. Protein in tempe flour using an oven is lower than tempe flour using freeze drying. The tempe used in this study came from the Indonesian Tempe House (RTI) in Bogor. This RTI was chosen because it uses a hygienic process and has an HCCP certificate so that food safety is maintained and there is no contamination during the manufacturing process. Tempe processed soybean products through a fermentation process causes an increase in total isoflavones, especially from aglycones which are much higher than tofu [34]. The food production process method, the type of yeast, and the type of soybean can also affect the nutritional content and isoflavones of tempe. In addition, isoflavones can be transformed into different conjugations, which have different effects based on food texture, bioavailability and pharmacokinetics on isoflavones [35,36,37]. Tempe contains protein, calcium, phosphorus, iron, vitamin A and B vitamins [38].

This study used tempe which were made in the form of flour using the freeze dry method. The choice of this form of flour is to increase the nutritional content and facilitate mixing homogenization when making drinks. The results showed that tempe flour with freeze drying contained isoflavones, namely genistein (41.21 mg/100g) higher than oven tempe flour (36.27 mg/100 g). Although statistically not significantly different (p> 0.05) so that the next product can use tempe flour with the oven method because the price is much cheaper. The results of the isoflavone content in this study were almost the same as those of Rahardjo et al. (2010)[39] which

showed that the isoflavone (genestein) content was 55,409 mg/100 g in tempe flour. Isoflavones in the study had a greater value than the results of the study by Safrida (2008)[40], the genistein content in tempeh flour was 25.065 mg/100 g body weight. The results of Aryani's research (2009)[41] found that the isoflavone (genetein) content of tempe was 38.9 mg/100 g. Research by Utari (2011)[42], the content of genistein in tempe is 30.8 mg/100 g. Differences in isoflavone content are due to differences in the process of making tempeh Tempe protein contains higher free amino acids by 30 to 35 times that of soybeans [43] and the results of research by Hermana and Karmini (1997)[22] increased free amino acids by 7.3% to 12%. This is because during fermentation by Rhizopus and bacteria it produces protease enzymes so that proteins are broken down into amino acids and peptides. The amount of amino acids released reached a peak after 24 to 72 hours of fermentation [44]. Increasing the release of amino acids will improve the nutritional value of tempe, where the protein digestibility corrected amino acid score (PDCAAS) is 0.8-0.9 or 80% - 90% of animal protein. During fermentation Rhizopus produces four groups of enzymes namely: lipase, protease, amylase and phytase. This enzyme is beneficial for individuals with digestive problems, and helps the digestion of proteins, fats and carbohydrates[22]. Phytase is also useful for reducing phytic acid from consumed vegetables thereby reducing minerals bound to phytic acid and increasing mineral bioavailability. The nutritional content (vitamins B3, B6, B12 and folic acid) in freeze drying tempe flour is higher than oven tempe flour. This happens because tempe flour uses an oven using ±60^{o} heating so that more nutritional content is lost compared to using freeze dry. Tempe nutrients are formed because during the manufacture of tempe, namely the soaking and fermentation stages, it causes acidic conditions resulting in bacterial growth for the synthesis of vitamin B2, vitamin B6, vitamin B12, niacin, biotin, folic acid, and pantothenic acid [22,45]. Vitamin B12 is generally obtained from animal protein. The fermentation process in soybeans can produce vitamin B12 due to the presence of contaminant bacteria, namely klebsiela pneumoniai in the lactic acid fermentation process. The process of lactic acid fermentation is not found in tempe produced in America or elsewhere. The process of lactic acid in the manufacture of tempe in America is carried out by adding lactic acid, so that in this situation it is suspected that the content of vitamin B12 is not present in tempe. Further research is needed to produce vitamin B12 which is not derived from the Klebsiella pneumoniae bacteria so that the vegetarian group can meet the needs of vitamin B12 by consuming tempe.

Hedogenic Test Results

The hedogenic test was carried out on 30 elderly residents of the Tresna Werdha Social Institution by measuring color, taste, aroma and thickness. Elderly consists of 51% of women and 49% of men with an age range of 60 years to 88 years. The hedogenic test results are shown in table 2 below:

Table 2. Average ± SD hedogenic test results include: color, aroma, taste and thickness

FORMULA	Color	Flavour	Taste	Thickness	Total
1	6±1.91	5.7±1.84	5.76±1.82	5.97±1.74	5.86±1.33
2	6.27±1.79	5.51±1.99	6.05±1.58	6.11±1.66	5.99±1.34
3	6.84±2.075	5.95±1.899	5.78±1.797	6.14±1.858	6.18±1.52

Based on table 2, it was found that the results of the organoleptic test on the preference level of tempe-based drinks. The results of the assessment of color preference, the highest value (6.84) is owned by formula 3 and the lowest (6) is owned by formula 1. The third formula is the formula most liked by respondents including color, aroma and thickness. The third formula is tempe flour using the freeze dry method with a thickness of 14%. The fingerprint of variance showed that the amount of water used had a significant effect ($P<0.05$) on the acceptance of the color of the tempe-based drink. The highest score for organoleptic viscosity was in formula 3 and the lowest score was in formula 1. For the organoleptic assessment of aroma, taste, color as a whole, the results of variance showed that the treatment had a significant effect ($P<0.05$) Respondents are elderly people between the ages of 60 and 88 years. It is intended that when this drink is given to the elderly, it is acceptable. However, it turns out that it is necessary to have a comparison of the pre-elderly and adolescent groups because if the parameters are only in the elderly, then there is a possibility that the results will be different from the younger age group. In the elderly over 60 years there is a possibility of numbness so that the results of the taste test can be biased. The results of the hedogenic test (favorability test) based on color, taste, aroma and viscosity were the highest or most preferred in the third formula, namely tempe drink with the addition of 1% chocolate as a flavor and with a concentration of 14%. This formula is not too thick so it is easy to swallow. Tempe-based drinks are given a 250 ml serving containing 27.16 mg of isoflavones. The nutritional content per serving is vitamin B3 (43.932 mg), vitamin B6 (0.1918 mg), vitamin folic acid (16.5025 mcg), and vitamin B12 (0.1036 mcg). Several studies of tempe in the elderly have been carried out. Seniors over 60 years of age who consume 75 g of tempeh every day have good cognitive function [41,16]. The effect of isoflavone administration is similar to that of estrogen administration in postmenopausal women aged 60 with Alzheimer's disease. Elderly people who consume tempe have better cognitive

function due to the possibility of the presence of vitamin B12 and folic acid in tempe [17]. Research File et al (2001) using 100 mg of total isoflavones given daily to subjects for 10 weeks, showed an increase in cognitive function in female subjects. According to Biben (1998)[19], 100 mg of total isoflavones is equivalent to 125 g of tempe (4-5 medium pieces) and 200 g of tofu (4 pieces). Isoflavones have a positive effect on cognitive performance and mood[46.]

CONCLUSIONS

Tempe flour using the freeze dry method contains isoflavones and nutrients (vitamin B3, vitamin B6, vitamin B12 and folic acid) higher than tempe using the oven method but not significantly. Hedogenic test (favorability test) is the highest with a 14% viscosity formula and there is the addition of chocolate.

REFERENCES

1. R C Petersen, R Doody, A Kurz, R C Mohs, J C Morris, P V Rabins, K Ritchie, M Rossor, L Thal, B Winblad. 2001. Current concepts in mild cognitive impairment. Arch Neurol. 58(12):1985-92
2. Rasyid A dan Dahlan P. 2000. Penyakit Alzheimer: prevalensi dan insiden. *B. NeuroSains.* 2(1): 221-225
3. Seriana van den Berg MD. 2012. Policy brief - Risk factors for dementia Alzheimer's Disease International . www.alz.co.uk
4. Lee Y. 2012. Systematic review of health behavioral risks and cognitive health in older adults. International psychogeriatrics. 22(02):174
5. 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
6. 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.
7. Fang Z, Carlson SH, Chen YF, Oparil S, and Wyss JM. 2001. Estrogen depletion induces NaCl-sensitive hypertension in female spontaneously hypertensive rats. *Am J Physiol Regulatory Integrative Comp Physiol.* 281:1934–9.
8. Karahalil B. 2006. Benefits and risks of phytoestrogen. Fatih Yildiz. Phytoestrogen in functional foods. USA: CRC Press. P.09-239
9. 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-con trolled trial. *American Society for Nutrition.* 84(2): 361-370.

10. M. Soni, L.R. White, A. Kridawati, S. Bandelow, E.Hogervorst. 2016. Phytoestrogen consumption and risk for cognitive decline and dementia: With consideration of thyroid status and other possible mediators (review). *The Journal of Steroid Biochemistry and Molecular Biology* 160: 67-77. Elsevier. 10.1016/j.jsbmb.2015.12.016.
11. Anna Maria Aloisi, Marco Bonifazi. 2006. Sex hormones, central nervous system and pain. Hormones and Behavior. Volume 50, Issue 1, June 2006, Pages 1-7
12. A Kridawati, A Sulaeman, R Damanik, A Winarto, T Rahardjo, E Hogervorst. 2013. Tempe and tofu may have a positive effect on cognitive function. *Annals of Nutrition and Metabolism* 63:213-2013
13. Atik Kridawati, Hardinsyah, Rizal Damanik, Ahmad Sulaeman, Adi Winarto, Tri Budi W. Rahardjo, Eef Hogervorst. 2016. Tempe Reversed Effects of Ovariectomy on Brain Function in Rats: Effects of Age and Type of Soy Product. *The Journal of Steroid Biochemistry and Molecular Biology* 160: 37-42. DOI 10.1016/j.jsbmb.2015.12.016
14. Atik Kridawati, Tri Budi, Hardinsyah, Rizal Damanik, Eef Hogervorst. 2019. Comparing the effect of tempe flour and tofu flour consumption on estrogen serum in ovariectomized rats. Heliyon 5: e01787
15. Atik Kridawati, Hardinsyah Hardinsyah, Ahmad Sulaeman, Tri Budi W. Rahardjo**,** and Eef Hogervorst. 2020. Tempe, Tofu, and Amyloid-_ 1–40 Serum Levels in Ovariectomized Rats. Journal of Alzheimer's Disease 76 (2020) 159–163
16. Hogervorst E, Sadjimin T, Yesufu A, Rahardjo T. 2008. High Tofu Intake is Associated with Worse Memory in Indonesian Elderly Men and Women. *Dementia.* 26:50-57
17. 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-con trolled trial. *American Society for Nutrition.* 84(2): 361-370.
18. File, Sandra E, Nicholas Jarret, Emma F, Rosanna Duffy, Karen Casey, Helen Wiseman. 2001. Eating soya improves human memory. *Psychopharmacology*. 157: 430-436.

19. Biben A. 2001. Effect of tempe dietary formula supplementation on pharmacy and bone resorption in pre- and post-menopausal women [Dissertation] Bandung (ID): Universitas Padjajaran
20. Purrwantyastuti. 2000. Relation of lipid peroxides to food habits, selected coronary heart disease risk factors and vitamin E supplementation the elderly [Disertation]. Jakarta (ID): University of Indonesia.
21. Karmini M, *et al*. 1997. The inhibitory effect of tempe on Escherichia coli infection. Reinventing the hidden miracle of tempe. Proceedings International Tempe Symposium, Juli 1997
22. Hermana and Karmini M. 1999. The development of tempe technology. Dalam "The complete handbook of tempe".*The American Soybean Association*.
23. Badan Pusat Statistik. 2007. *Penyusunan data sosial ekonomi Daerah Provinsi Jawa Barat.* Jawa Barat (ID): BPS
24. Silitonga C dan Djanuardi B. 1999. Tempe consumption pattern, in the complete handbook of tempe. The American Soybean Association, Singapore
25. Ferri, C. P., Prince, M., Brayne, C., Brodaty, H., Fratiglioni, L., Ganguli, M., et al. 2005. Global prevalence of dementia: a Delphi consensus study. Lancet, 366(9503), 2112-2117
26. Bhanthena, Manual T Velasquez. 2002. Beneficial Role of Dietary Phytoestrogen in Obesity and Diabetes. *Am j Clin Nutr*. 76:1191-1201
27. Kuiper GG, Shughrue PJ, Merchenthaler I, Gustafsson JA, 1998. The estrogen receptor beta subtype: a novel mediator of estrogen action in neuroendocrine systems. *Frontiers in neuroendocrinology*. 19:253-286
28. Barnes S, Peterson TG. 1995. Biochemical Targets of the Isoflavone Genistein in Tumor Cell Illess. *Proc Soc Exp Biol Med*. 208:103-8
29. Eef Hogervorst, Stephan Bandelow. 2010. Sex steroids to maintain cognitive function in women after the menopause: A meta-analysis of treatment trials. *Maturitas* 66, Issue 1: 56-71. https://doi.org/10.1016/j.maturitas.2010.02.005
30. Mira Soni, Tri Budi W. Rahardjo, Rodiyah Soekardi, Yenny Sulistyowati, Lestariningsih, Amina Yesufu-Udechuku, Atik Irsan, Eef Hogervorst. 2014. Phytoestrogens and

cognitive function: a review. Maturitas, 77(3):209-20

31. White LR, Petrovitch H, Ross GW, et al. 2000. Brain aging and midlife tofu consumption. J Am Coll Nutr 2000;19:242–55.
32. McClements D. Julian. 2007. Analysis of Proteins. University of Massachusetts Amherst.
33. Amidzic Rada, Arboric J. Jasmin, ^Udina Olivera and Vladimirov Sote. 2005. RP-HPLC Determination of vitamins B1, B3, B6, folic acid and B12 in multivitamin tablets. *J. Serb. Chem. Soc.* 70 (10):1229–1235.
34. 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
35. Villares A, Rostagno MA, Lafuente AG, Guillamon E, Martinez JA. 2011. Content and profile of isoflavones in soy- based foods as a function of the production process. Food and Bioprocess Technology [serial on the Internet]. January [cited 2013 Feb 26]; 4 (1): p. 27-38
36. Cai, T. and K.C. Chang. 1999. Processing effect on soybean storage proteins and their relationship with tofu quality. *J. Agric. Food Chem*. 47: 720−727
37. Mujoo R, Trinh DT, Ng PKW. 2003. Characterization of storage protein in different soybean varieties and their relationship to tofu yield and texture. *Food Chem*. 82(2): 265-73
38. Food Composition Data (DKBM). 2018. Ministry of Health of the Republic of Indonesia.
39. 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
40. Safrida. 2008. Changes in Estrogen Hormone Levels in Rats Given Soybean Flour and Tempeh Flour [Thesis]. Bogor (ID): Institut Pertanian Bogor.
41. M.F. Aryani. 2009. Relationship between Tempe and Tofu Consumption with Elderly Cognitive Function [Desertation], Universitas Indonesia.
42. Utari DM. 2011. Effect of tempeh intervention on lipid profile, superoxide dismutase, oxidized LDL and malondialdehyde in menopausal women [Dessertation]. Bogor (ID): Institut Pertanian Bogor.
43. Kiers Jeroen L. 2001. Effects of fermented soya bean on digestion, absorption and diarrhea [Disertation]. Wageningen:

Wageningen Universiteit , Netherland.

44. 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.
45. Pawiroharsono 2007 Pawiroharsono S. 1999. Microbiological Aspects of Tempe. The complete handbook of tempe, the unique fermented soy food of Indonesia. *The American Soybean Association.*
46. Rishi, R.K., 2006 Chemistry and Mechanism Of Action Of Phytoestrogens. dalam Yildiz, F. Phytoestrogen in Functional Foods (pp. 81-95). USA. CRC Press.