Food Science and Engineering
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Review

Proposed Design and *In-silico* Studies of Functional Idli as a Prophylactic Measure to Reduce Post-Menopausal Adverse Effects in Women

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Received: 27 May 2023; **Revised:** 25 September 2023; **Accepted:** 8 October 2023

Abstract: Food Science and Engineering have become a rapidly evolving domain that has gained prominence in recent years to facilitate the development of enriched food products (like functional foods). It is utilized as a prophylactic measure for several health conditions like osteoporosis, obesity, cardiovascular diseases, and so on. Functional foods perform prophylactic activity through components like phytochemicals in their ingredients which may interact with the target receptors in susceptible people. The post-menopausal women become susceptible to numerous complications, including bone atrophy, osteoporosis, depression, dementia, obesity, vasomotor symptoms, and other lifestyle disorders. As reduced estrogen production is the major reason for such conditions' development, therapeutic interventions like hormone replacement therapy are administered to treat the complications associated with post-menopause. Hormone therapy is identified to be associated with some important limitations. This gives rise to the need to develop safer approaches for prophylaxis of post-menopausal adverse. In this review, we attempt to propose a design of functional idli made up of oats (*Avena sativa*), soybean (*Glycine max*), decorticated black gram (*Vigna mungo*), rice (*Oryza sativa*), moringa leaves (*Moringa oleifera*), spinach (*Spinacia oleraceae*) and fenugreek (*Trigonella foenum-graceum*) as a prophylactic measure to reduce post-menopausal adverse effects in women. These ingredients are sources of phytoestrogen (that structurally mimics human estrogen), consumption of which is beneficial in promoting healthy life in post-menopausal women. Computational studies like molecular docking can be utilized for *in-silico* validation of the efficacy of the formulation by demonstrating the effective binding of the phytoestrogens with the estrogen receptors α and β.

***Keywords*:** functional food, idli, molecular docking, phytoestrogens, post-menopause

1. Introduction

The domain of food engineering has rapidly gained prominence in recent years and has notably influenced food systems from production to consumption stages. It includes the design and development of food with properties like nutrient supplementation and preservation, removal of toxins, disease management and prophylaxis, as well as the reduction in wastage or product loss [1]. Food engineering is widely used to design functional foods that exhibit advantageous physiological properties to promote human health and lower the risks of disease development, in

DOI: https://doi.org/10.37256/fse.4220233121

addition to its basic nutritional supplies [2]. The development of functional foods is greatly dependent on the "scientific intelligence" in food preparation which involves understanding the health benefits of the ingredients used [3]. Functional foods can also be designed as a prophylactic measure to reduce adverse effects associated with post-menopause.

Menopause refers to the end of the menstruation phase as a result of reduced ovarian function and deficiency of female sex hormones [4]. It is a phase in natural biological aging [5], caused by a deficiency of the hormone estrogen [6], attributed to the reduction in the activity of ovarian follicles [7]. The reduction in estrogen levels is associated with vasomotor functions leading to increased susceptibility to metabolic disorders, cardiovascular diseases, bone loss, and atrophy [4, 7]. It is also accompanied by changes in sleep habits, diet, mood, and other lifestyle elements [4]. It may also lead to the development of conditions such as osteoporosis, osteoarthritis, reduced cognitive activity, dementia, depression, tiredness, anxiety, sadness, hypersensitivity, and irritation [8-10]. Vasomotor symptoms including hot flashes and night sweats are the most common symptoms prominent in over 80% of women [11]. These are caused by vasodilation of blood vessels located near the skin epithelium. This condition is prominent in women with higher body fat percentages or body mass index [12]. Studies revealed that it can also lead to the development of various types of cancers in women [8]. The conditions and increased risks associated with post-menopause are presented in Figure 1.

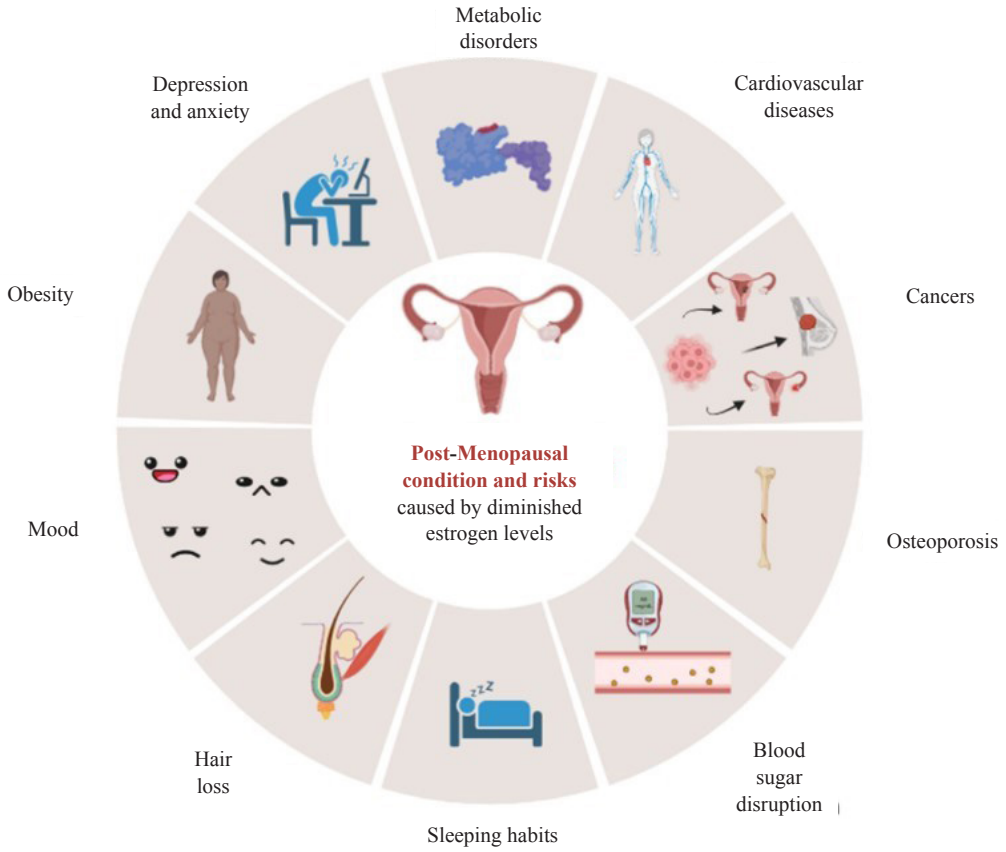


Figure 1. Conditions and risks associated with post-menopause

The median age of menopause, according to studies, is 51 years. In the United States, the average age of women experiencing menopause was found to be around 51 years [6], whereas globally the average range of age was found to be between 45 to 55 years [5]. The majority of women reportedly experience menopause in the age group of 49 to 52 years. Cases, where women experience menopause before the age of 40 years are termed premature menopause [13]. Studies have revealed that the post-menopausal symptoms reported by Asian women are significantly lower compared to the rest of the world [14]. In the context of India, it was identified that over three fourth of women experienced the

symptoms of menopause like hot flushes, depression, and sweating in which approximately two-thirds of the population exhibited the poorer quality of life [15].

The health condition of post-menopausal women is essentially dependent on socio-demographic, genetic, lifestyle, cultural, behavior, and diet-dependent factors [16-17]. With increasing age, women after menopause become susceptible to breast, uterine, and ovarian cancers which are associated with the hormone, estrogen [18]. It is also observed that the molecular features and risk factors of breast cancer in postmenopausal women are different from pre-menopausal women [19]. Before menopause, estrogen is secreted from ovaries and fat tissues in a female, but after menopause, the ovaries stop the secretion of estrogen and the production happens only by fat tissues [20]. After menopause, estrone is the dominant estrogen synthesized actively by adipose tissue which is reported to increase the risk of obesity, metabolic disorders, and cancers [4].

This review discusses the commonly used therapeutic interventions for the prevention and treatment of post-menopausal conditions and their limitations. The study also attempts to propose a design for functional idli made using soybean, oats, black gram, rice, spinach, moringa, and fenugreek, as a safer approach to reduce post-menopausal complications, promote good health and improve the quality of life. The efficacy of the functional idli is analyzed and validated using in-silico techniques like Lipinski's rule of five analysis and molecular docking studies of phytoestrogens (present in the components of idli) with estrogen receptors α and β.

2. Therapeutic approaches

The therapeutic approaches for the treatment of post-menopausal complications are categorized into hormonal and non-hormonal types. Hormone therapy, also known as hormone replacement therapy comprises of administration of sex hormone preparations in patients with deficiency. In the case of post-menopause, the affected women show a deficiency in the level of the hormone estrogen which can be treated by supplementation of the hormone using hormone therapy. This approach is called estrogen replacement therapy as it involves only the estrogen hormone [21]. The estrogen therapies usually involve human estrogens like estradiol, estriol, and estrone as well as estrogen derived from animals like conjugated equine estrogen and synthetic estrogens such as ethinyl estradiol [22-23]. The Food and Drug Administration (FDA) indication for both of these hormones is identical, despite their distinct implications on the human body. The hormones are administered as oral or transdermal preparation due to easy absorption by skin, mucus membranes and gastrointestinal tract. The oral administration leads to raised resistance of activated protein-C, risk of blood clotting and cause rupture of atherosclerotic plaque. The transdermal administration is via cream, subdermal pellets, vaginal patch and vaginal inserts. It results in bypassing the activation of protein-c resistance as well as decreasing the risks of development of blood clots [22]. The estrogen only therapy is administered to women who had undergone hysterectomy, whereas, the combination of estrogen and progesterone is administered to women with uterus intact [23]. Studies have reported that administration of the hormonal intervention to women below 60 years of age reduces mortality due to all associated complications like cardiovascular disorders, coronary heart diseases, etc. [24]. It is recommended to personalize the hormone replacement therapy based on the personal details and factors involved for each patient [11]. The estrogen hormonal theory is often provided in combination with progesterone. Hormonal therapy facilitates reduction in the risks of developing bone fractures, cancers (gastric, colorectal, and esophageal), as well as diabetes mellitus [25]. In contrast to this, the non-hormonal therapies include the use of some antidepressants (like selective serotonin reuptake inhibitors and serotonin-norepinephrine uptake inhibitors), clonidine, gabapentinoids, as well as oxybutynin which reportedly provides relief from hot flashes. There is another therapeutic approach with high efficiency in menopausal women. It is called cognitive behavioral therapy that has positive implications on the vasomotor symptoms. Studies have revealed that there is a lack of awareness among women in the aspects of available therapies to provide relief from the symptoms and reduce the side effects and enhance the quality of life [11]. The therapy targets vasomotor symptoms, low mood, sleep issues and stress. It is a short therapy that is conducted for around four to six sessions and is notably dependent on evidences and theory. The therapy considerable reduce the impact of vasomotor symptoms and improve the quality of life of the women experiencing it. Studies have shown consistent results when the therapy is provided in groups, via self-help books in addition to online formats and content. The cognitive behavioural therapy is recommended by the North American Menopause Society for women experiencing depression and anxiety during the post-menopausal condition. The telephonic form of therapy is efficient in treating

insomnia [26].

Hormone therapies are associated with limitations such as inability to explain the variations in the factors responsible including the type, dosage, duration, composition of the formulation, mode of administration, as well as the age at which a women experience the beginning of menopause. The hormone therapy, also enhances the probability of developing venous thromboembolism, cancers of breast and ovary in addition to stroke, and disease of gall bladder [25]. It also increases the risk of cardiovascular conditions [27]. On the other hand, non-hormonal therapies are associated with limitations such as side effects on menopausal women, lower efficiency than hormonal therapy, as well as variations in efficacy from patient to patient [11]. Therefore, arises the need to design an approach that is efficacious and safer compared to the available options. An important approach for this can be the use of functional foods supplemented with elements that enhance the health and reduces the risk of development of complications associated with post-menopause. Certain dietary plans can facilitate reduction in body weight as well as vasomotor symptoms in the post-menopausal women [12].

3. Functional food for post-menopause

Proper nutrition and diet can be efficient in reducing the adverse effects of post-menopausal complications, consequently enhancing the quality of life for women experiencing it. Foods rich in antioxidant as well as anti-inflammatory properties significantly reduces blood pressure, fat and regulate cholesterol levels. The fat mass can be efficiently reduced by uptake of a plant-based diet constituting of fruits, vegetables and grains [7]. The dietary supplementation with functional foods enriched with isoflavones significantly altered the intestinal microbiota of women in post-menopause which had positive implications on their health. This supplementation can sufficiently prevent the development of post-menopausal complications [28]. Studies have reported food items like broccoli, soybean, flaxseeds, oats, finger millet, Moringa, amaranth, red clover, bugbane, and evening primrose that are beneficial in regulating the post-menopausal conditions [29].

Plants are rich in phytochemicals called phytoestrogens that can efficiently mimic the activity of estrogen in women's bodies. Therefore, post menopause, when they suffer from estrogen deficiency, phytoestrogen rich foods can be provided as supplements to improve the symptoms as well as quality of life. A study performed in Egypt on perimenopausal women, to analyze the efficiency of dietary supplements comprising peanut, sesame, coriander seeds and thyme, demonstrated the efficiency of the formulation in improving psychological, somatic and urogenital symptoms [30]. Dairy proteins have positive health implications in post-menopausal women. A study by Durosier-Izart et al., revealed that the intake of proteins derived from dairy and animal sources promotes bone strength and improves the microstructure in post-menopausal women. Consequently, it reduces the risk of fractures [31]. The Iowa Women's Health Study aimed to identify the correlation between the consumption of whole grain fibers with the mortality risks. It reported that the post-menopausal women with a daily intake of 1.9 g of refined grain fibers along with 4.7 g of whole grain fibers exhibited 17% reduced mortality risks than those with a higher intake of refined fibers than the whole grain fibers [32]. The whole grains are rich source of phytochemicals and nutrients comprising of phenolic compounds, bioactives, vitamins, minerals and lignans that are beneficial for the health of post-menopausal women. The dietary uptake of wholegrains reported reduces the probability of cardiovascular diseases and diabetes mellitus by 20-30% [33].

Studies also reveal that foods rich in phytoestrogens like soyabean, spinach, beans, lentils, etc., significantly reduce the occurrence of symptoms like hot flashes in women with no serious adverse effects [34-35]. Phytoestrogens comprise of flavonoids (which include isoflavones, flavones, and coumestans) and non-flavonoids (which includes lignans and derivatives of resorcinol). Soybean and other sources, such as fenugreek, blackcurrant, wheat, ginseng, berries, and sesame seeds are major sources of phytoestrogen [36] for post-menopause.

4. Health benefits of Indian foods like idli

Indian cuisine includes an array of traditional functional foods that are beneficial in preventing a wide range of illnesses and problems, in addition to being a significant source of nourishment [37]. Traditional Indian foods are predominantly plant-based and contain functional molecules like chemicals that induce healing, dietary fibers,

antioxidants, and probiotics. These chemicals support healthy weight management, blood sugar regulation, and immune system enhancement [38].

The food products like idli and dosa are found to boost probiotic activity [39]. Idli (steamed rice cakes) is a popular South Indian breakfast food, which provides a balanced diet consisting of carbohydrates and proteins. They have a high nutritional profile and are easy to digest. As a result, these are preferred by people from all age groups including patients [40]. It is usually prepared by fermentation of rice (*Oryza sativa*) and decorticated black gram (*Vigna mungo*) by the microorganisms Leuconostoc mesenteroides and Streptococcus thermophilus [41], Streptococcus faecalis, Lactobacillus fermentum, and Saccharomyces cerevisiae are some of the other microorganisms responsible for the fermentation of the batter [42]. These microorganisms facilitate the leavening of the idli batter by producing lactic acid and carbon dioxide, which increases the volume of the batter. The quantity of Vitamin B1, B2, B9, and C, free sugar, free nicotinic acid, methionine, and choline rises during the overnight fermentation [43].

$$\text{Idli Batter + Microorganisms} \xrightarrow{\text{Fermentation}} \text{Lactic acid + } CO_2 \text{ + Increase in volume}$$

Studies reveal that the raw materials or ingredients of idli contribute as a source of microorganisms for fermentation in addition to being utilized as a substrate for fermentation [42-43].

5. Functional food designed for post-menopause

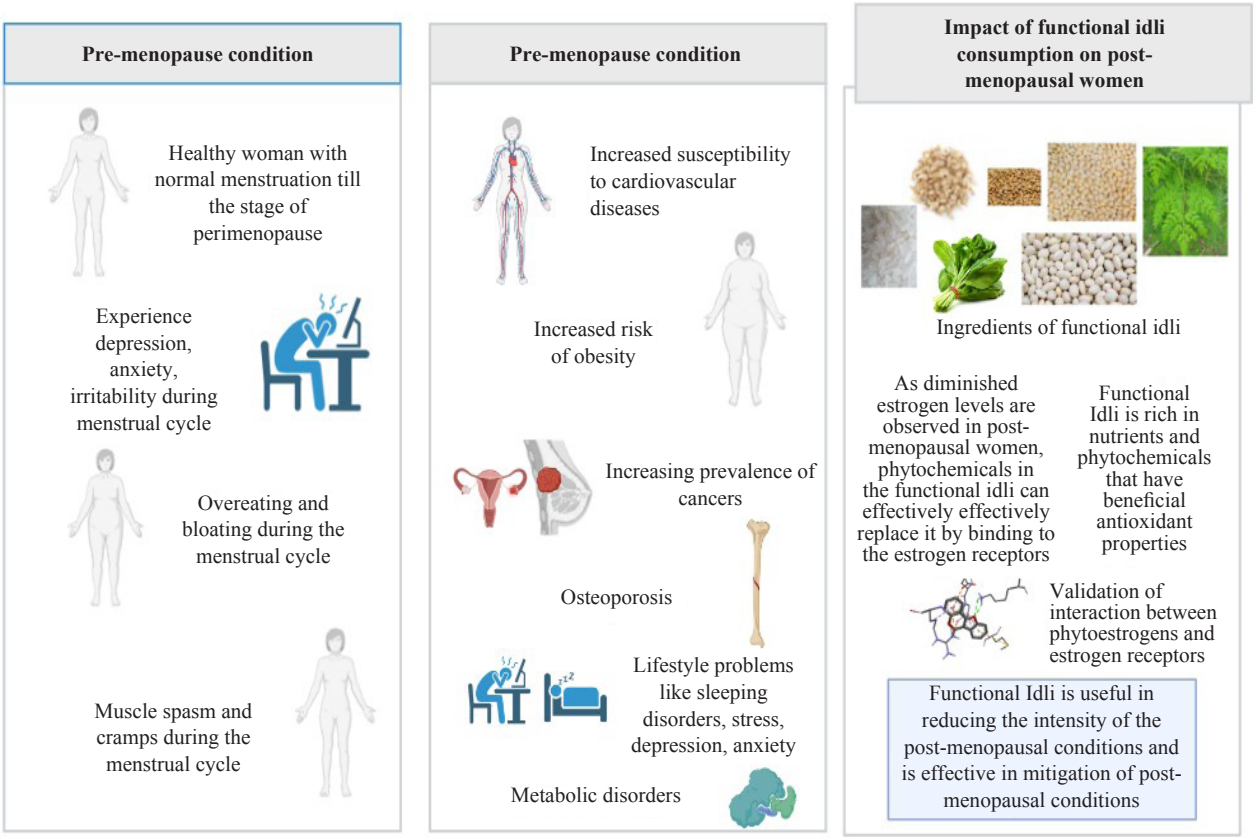


Figure 2. Symptoms of post-menopausal conditions and impact of functional idli in its prophylaxis

A functional idli can be prepared by supplementing it with ingredients that promote post-menopausal health. We formulate a functional idli for post-menopausal condition using oats (*Avena sativa* L.), soybean (*Glycine max*), decorticated black gram (*Vigna mungo*), rice (*Oryza sativa*), moringa leaves (*Moringa oleifera*), spinach (*Spinacia oleraceae* L.) and fenugreek (*Trigonella foenum-graceum* L.). These ingredients are rich in bioactives and nutrients that are reportedly to be beneficial for menopausal and post-menopausal women. Also, the functional idli prepared has high protein content that help in improving bone strength and muscle mass. Figure 2 presents an overview of the utility of functional idli as a prophylactic measure for post-menopausal condition.

5.1 *Preparation of functional idli*

The functional idlis can be prepared by taking 120 grams, 100 grams, 25 grams, 200 grams, 50 grams, 25 grams and 3 grams of the ingredients including soybean, black gram, oats, rice, spinach, moringa leaves, and fenugreeks, respectively. Soybean, rice, black gram, and fenugreek seeds are soaked overnight in separate containers. Following which they are ground in a mixer grinder along with spinach and moringa leaves to obtain a batter of flowing consistency. The preparation is mixed well and 3.2 grams of salt is added to facilitate fermentation that further enhances the health profile of the functional idli. The batter is kept for fermentation for a period of 8-12 hours. The batter then is ready for the preparation of idli by adding it in for idli mould and steaming in a steamer.

5.2 *Lipinski's rule of five analysis*

The Lipinski rule of five is a physio-chemical analysis to determine the drug-likeness of a compound. Software designed by IIT Delhi (http://scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) can be used for the analysis of Lipinski rule of Five for the key bioactives present in the raw materials of idli.

Table 1. Lipinski rule of Five physio-chemical analysis of phytochemicals

Phytoestrogen	Molecular mass	Hydrogen bond donor	Hydrogen bond acceptor	LogP	Molar refractivity
Apigenin	270	3	5	2.419	70.813
Luteolin	286	4	6	2.125	72.478
Genistein	270	3	5	2.419	70.813
Daidzein	254	2	4	2.713	69.149
Kaempferol	286	4	6	2.305	72.385
Quercetin	302	5	7	2.010	74.050
Myricetin	318	6	8	1.716	75.715
Vanillic acid	168	2	4	1.099	41.618
Caffeic acid	180	3	4	1.195	46.441
Ferulic acid	194	2	4	1.498	51.328
p-coumaric acid	164	2	3	1.490	44.776
Coumestan	236	0	3	3.632	66.608

The data from the Lipinski rule of Five analysis (as presented in Table 1) indicate that all the selected phytochemicals pose drug-likeness by fulfilling a minimum of two or more criteria or rules. It was observed that all phytoestrogens analyzed in this study exhibit maximum likeness by fulfilling all five rules, except myricetin that doesn't fulfil the criteria of less than 5 hydrogen bond donors. Thus, the phytochemicals discussed are efficient in prophylaxis of post-menopausal conditions caused by enhanced level of estrogen.

5.3 *Molecular docking*

5.3.1 *Preparation of ligand structures*

The structures of ligands are downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) [44] in Structured Data Files (SDF) format (Figure 3). These ligands possess estrogenic properties. The structures in SDF format are converted to Protein Data Bank (PDB) format using the Open Babel GUI application (http://openbabel.org/) [45]. The torsion trees of the ligand are detected and selected, preparing it for docking analysis using AutoDock tools. Table 2 reports phytoestrogens present in the ingredients of the functional idli.

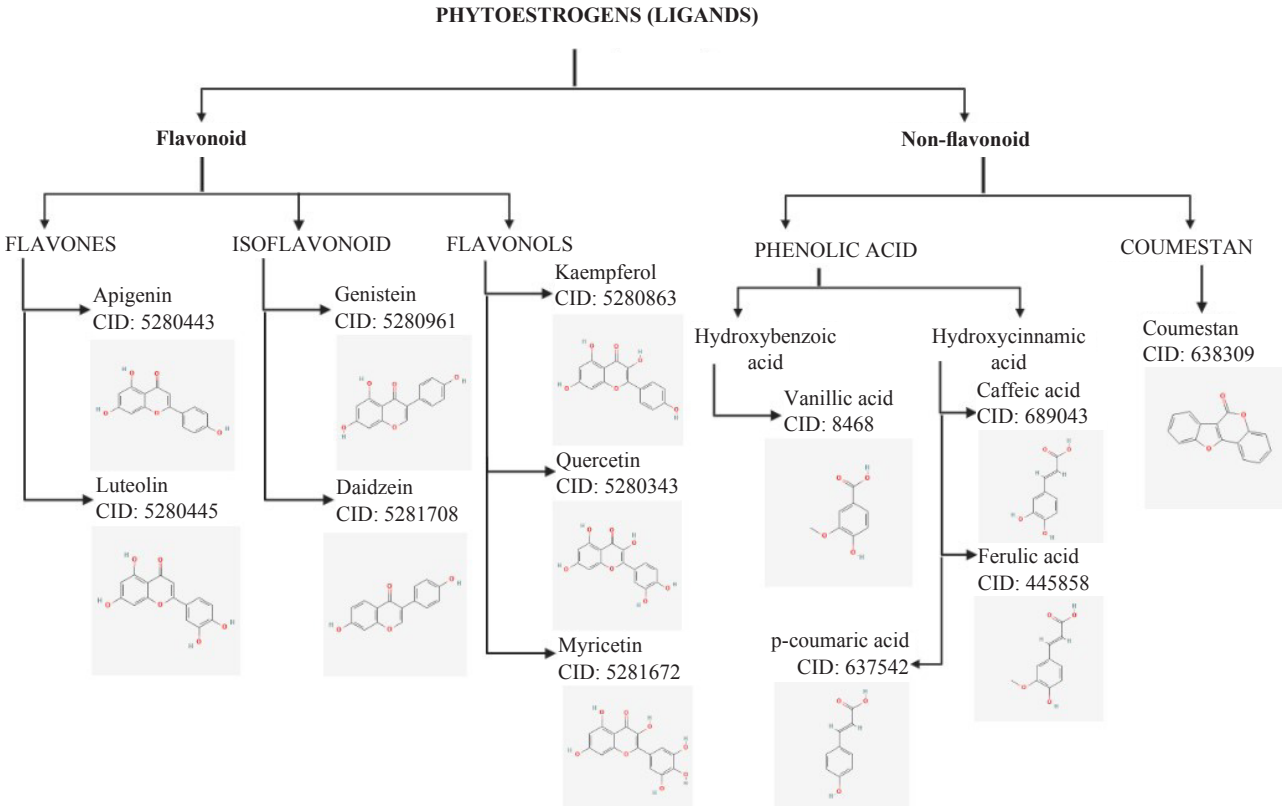
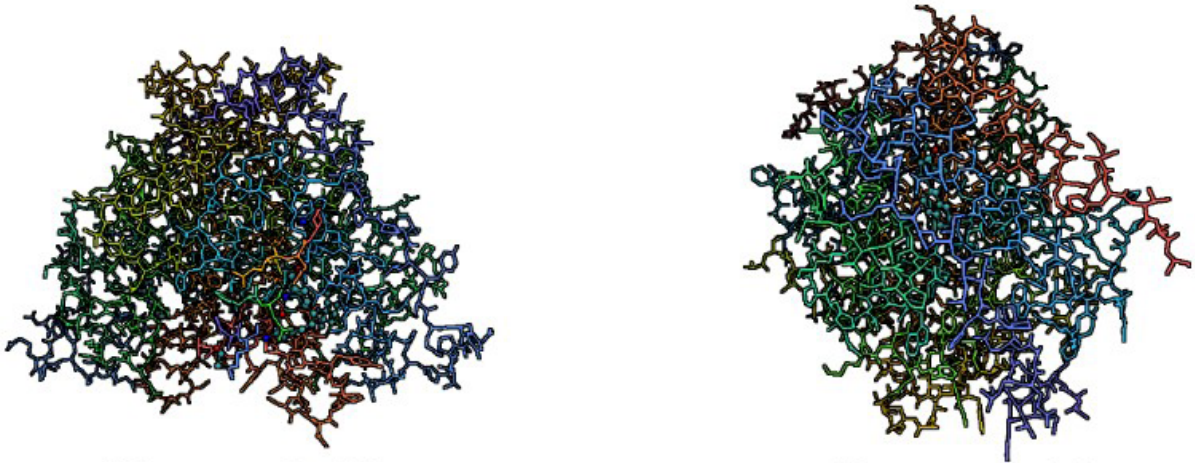


Figure 3. Classification of Phytoestrogen (ligands)

Table 2. Phytoestrogens in ingredients of functional idli

Phytoestrogens	Ingredients	References
Apigenin	Soybean, spinach, oats	[46-48]
Luteolin	Soybean, spinach, oats, fenugreek, rice	[36, 46-49]
Genistein	Soybean, spinach, black gram, moringa	[46-47, 50-51]
Daidzein	Soybean, spinach, black gram, moringa	[46-47, 50-51]
Kaempferol	Spinach, oats	[46, 48]
Quercetin	Oats, fenugreek	[36, 48]
Myricetin	Spinach	[46]
Vanillic acid	Oats	[48]
Caffeic acid	Oats	[48]
Ferulic acid	Spinach, oats	[46, 48]
p-coumaric acid	Spinach, oats	[46, 48]
Coumestan	Soybean, black gram	[47, 50]

5.3.2 *Preparation of receptor structures*



Estrogen receptor alpha
(2YJA)

Estrogen receptor beta
(5TOA)

Figure 4. Molecular structures of the receptors

The target receptor or protein for the ligands are Estrogen receptors α and β. The PDB structures of the ligands are

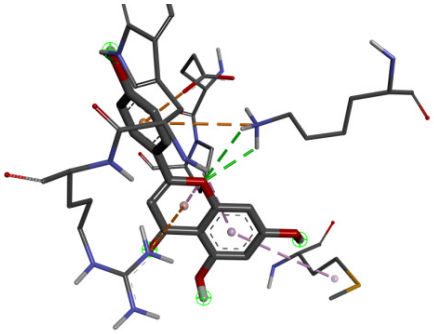
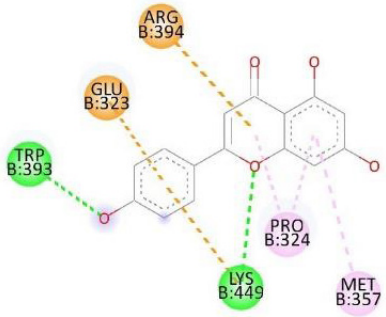
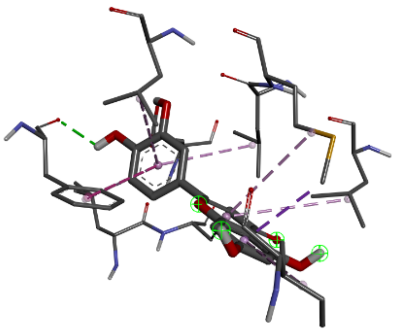
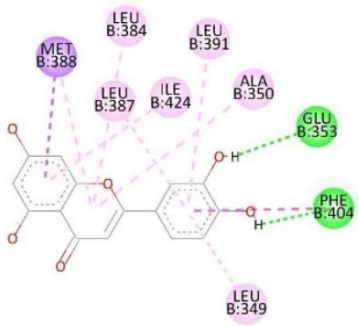
downloaded from RCSB PDB (https://www.rcsb.org/) [52] with the PDB ID 2YJA and 5TOA for estrogen receptors α and β, respectively. The receptor structures are prepared for docking using AutoDock tools by removal of water molecules, hetero atoms, and bound ligands. The polar hydrogen atoms are added followed by the addition of Kollman charges and the computation of Gasteiger charges. The molecular structures of the receptors are presented in Figure 4.

5.3.3 *Docking analysis*

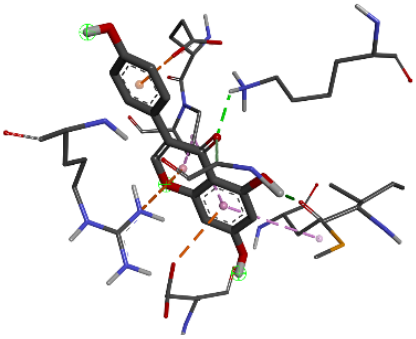
The docking analysis is performed using AutoDock tools and AutoDock vina application (https://vina.scripps.edu/) [53]. The docking parameters for prepared receptors and ligands are selected in AutoDock tools in addition to setting the grid box for blind docking, followed by running the program in AutoDock vina and Vina split applications. The analysis is further performed using the application Discovery BIOVIA Studio (https://www.3ds.com/products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/).

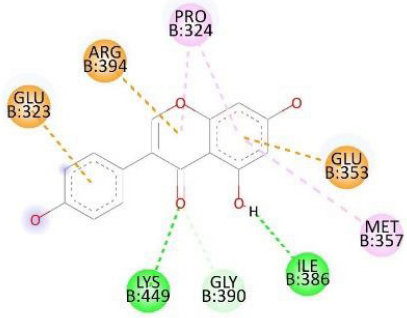
The approach of molecular docking is widely used for drug designing because it offers easy identification of ligands (drug molecules) for the target receptor. This in-silico technique has facilitated specificity, accuracy, and efficacy in designing and developing drugs. Using AutoDock tools and AutoDock vina, the estrogen receptors α and β (target receptors) were bound to isoflavones (phytoestrogens) present in the functional idli, that mimic 17β-estradiol and effectively bind with estrogen receptors. The interaction between the receptors and isoflavones is presented in Table 3.

Table 3. The interaction between the ligands (phytoestrogens) with estrogen receptors

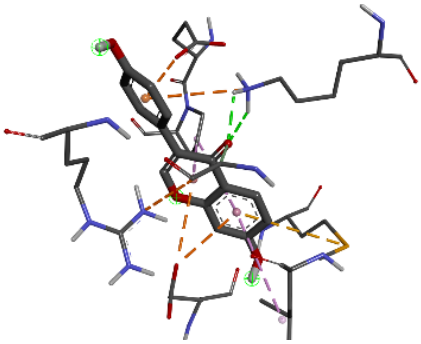
Ligand	3D interaction	2D interaction
	Estrogen receptor α (PDB ID: 2YJA)	
Apigenin (Binding affinity: -4.6 kcal/mol)		
Luteolin (Binding affinity: -1.2 kcal/mol)		

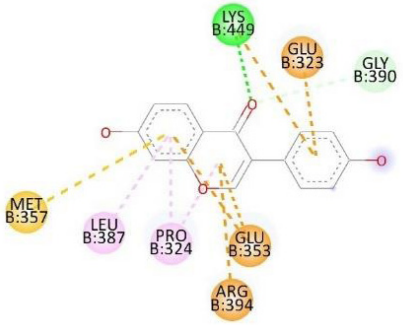
Genistein
(Binding affinity:
-4.7 kcal/mol)



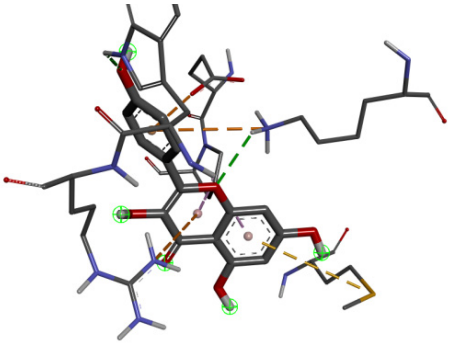


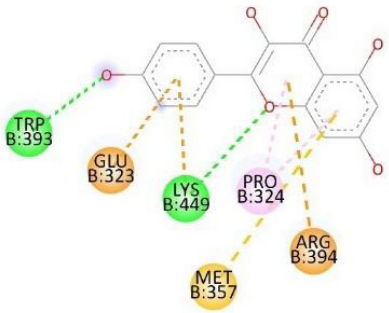
Daidzein
(Binding affinity:
-4.5 kcal/mol)



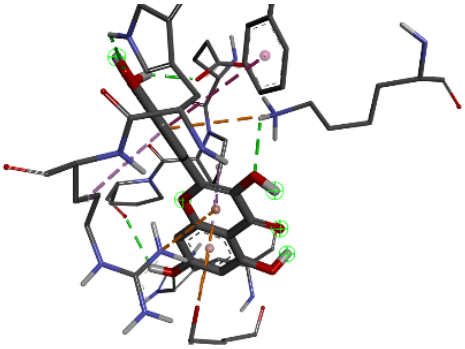


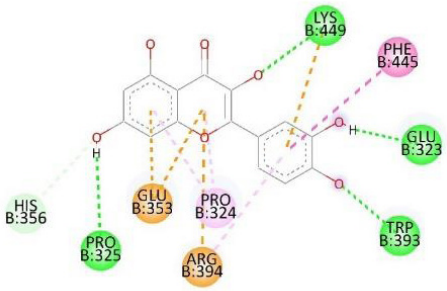
Kaempferol
(Binding affinity:
-4.8 kcal/mol)



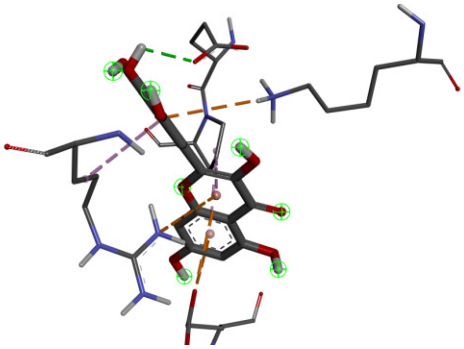


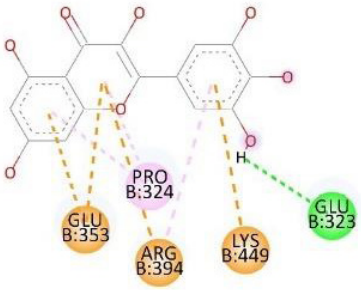
Quercetin
(Binding affinity:
-4.6 kcal/mol)



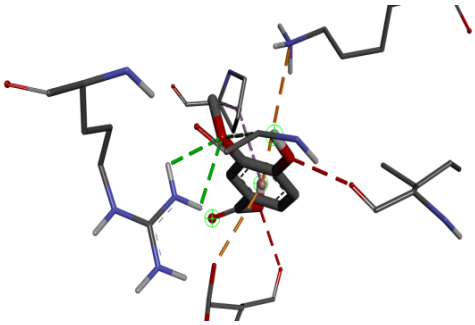


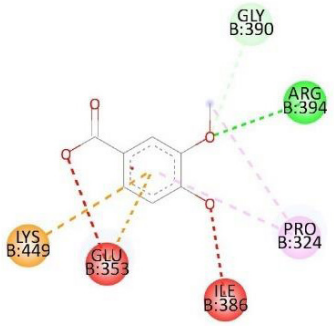
Myricetin
(Binding affinity:
-3.3 kcal/mol)



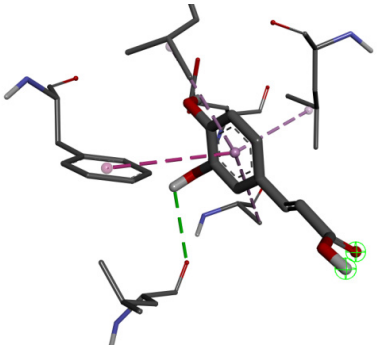


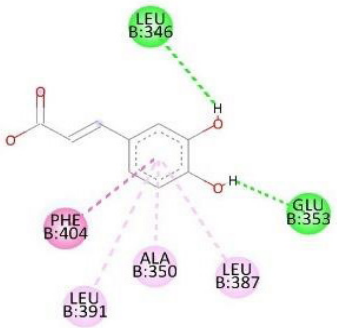
Vanillic acid
(Binding affinity:
-5.9 kcal/mol)



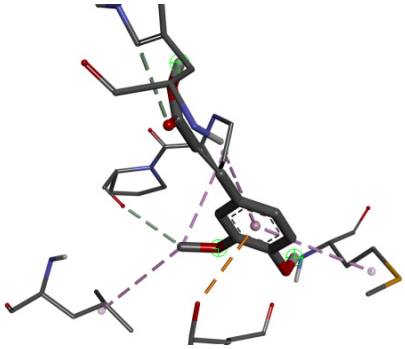


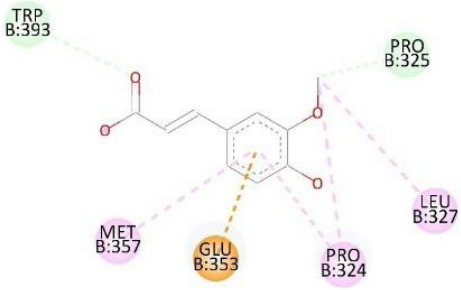
Caffeic acid
(Binding affinity:
-6.3 kcal/mol)



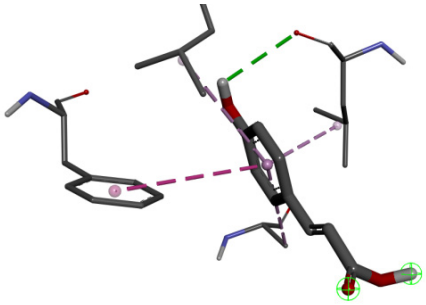


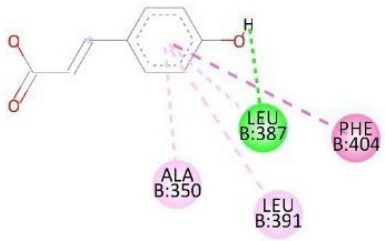
Ferulic acid
(Binding affinity:
-6.0 kcal/mol)



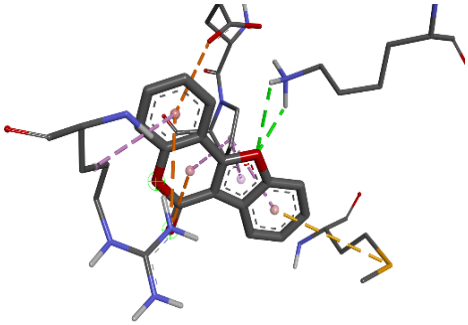


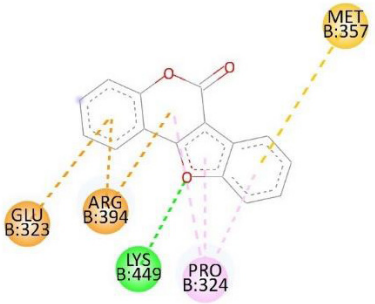
p-coumaric acid
(Binding affinity:
-5.9 kcal/mol)





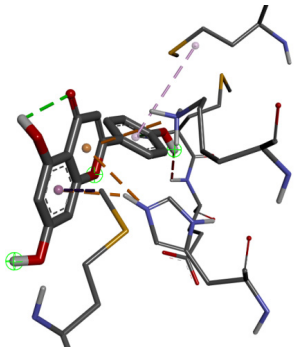
Coumestan
(Binding affinity:
-8.0 kcal/mol)

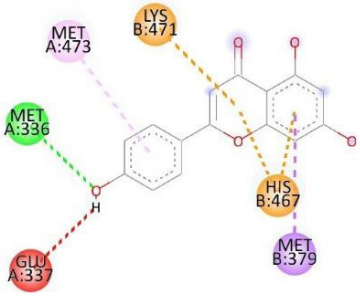




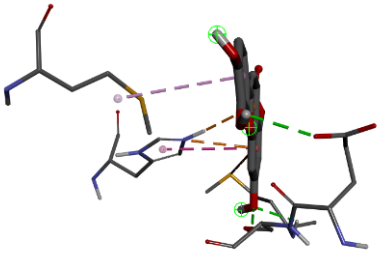
Estrogen receptor β (PDB ID: 5TOA)

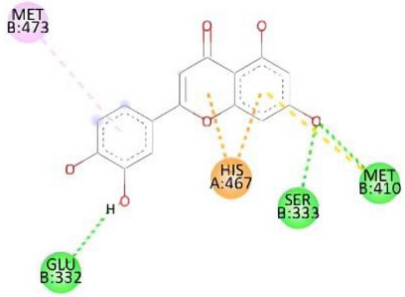
Apigenin
(Binding affinity:
-4.0 kcal/mol)



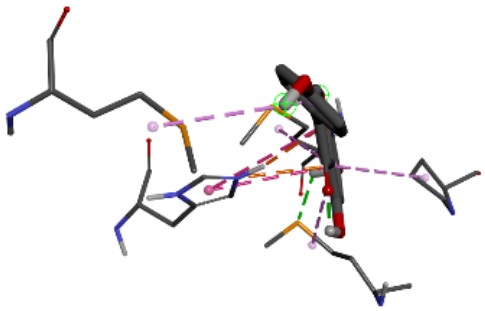


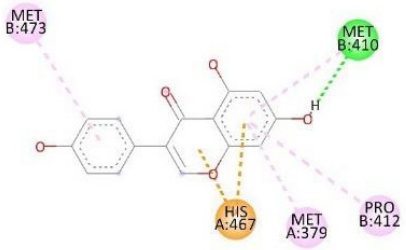
Luteolin
(Binding affinity:
-4.7 kcal/mol)



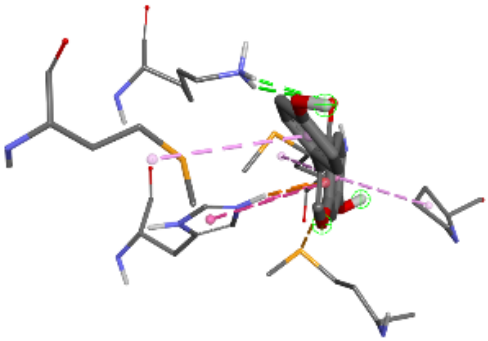


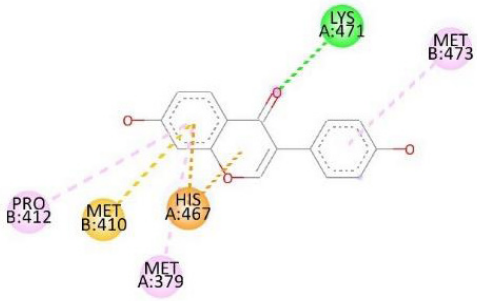
Genistein
(Binding affinity:
-3.5 kcal/mol)



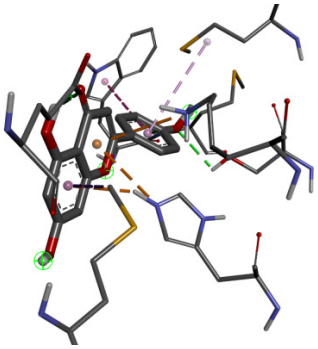


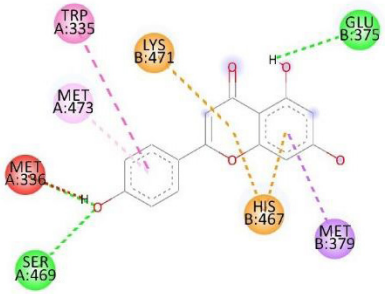
Daidzein
(Binding affinity:
-3.7 kcal/mol)



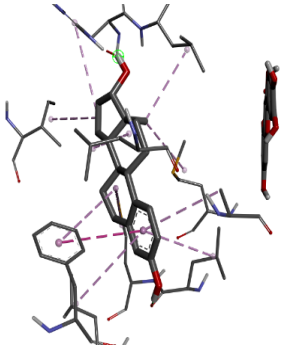


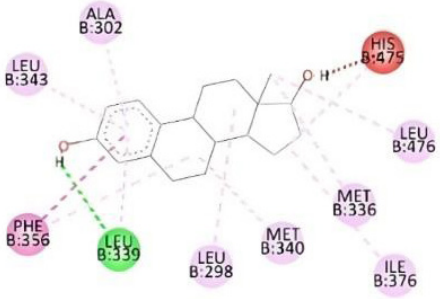
Kaempferol
(Binding affinity:
-4.0 kcal/mol)



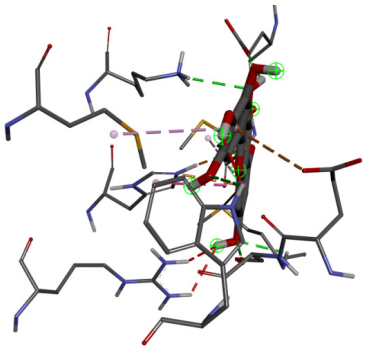


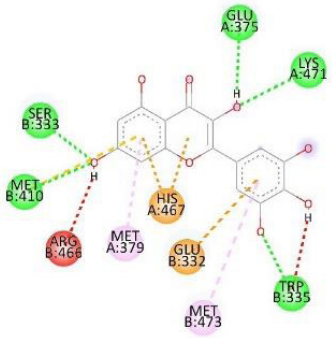
Quercetin
(Binding affinity:
-5.2 kcal/mol)



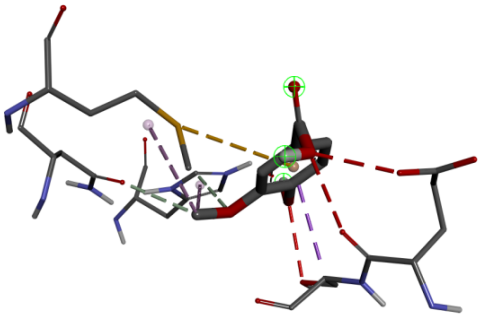


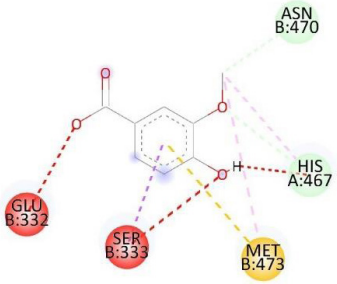
Myricetin
(Binding affinity:
-3.4 kcal/mol)



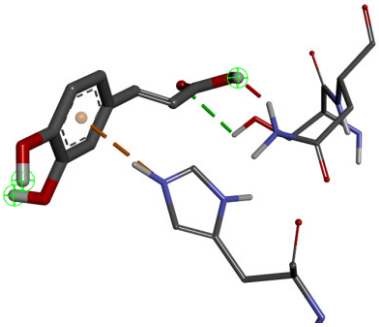


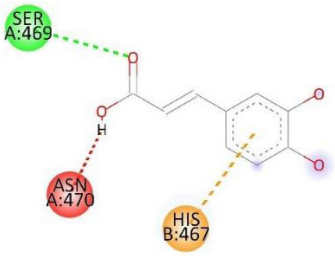
Vanillic acid
(Binding affinity:
-5.0 kcal/mol)



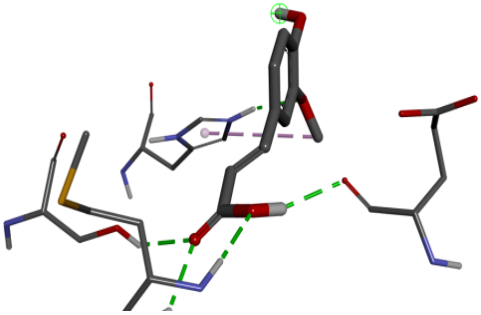


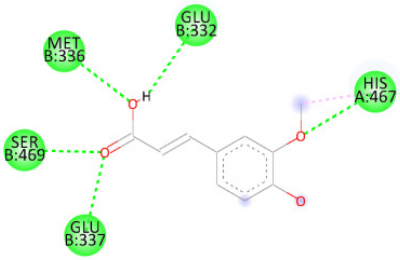
Caffeic acid
(Binding affinity:
-4.9 kcal/mol)



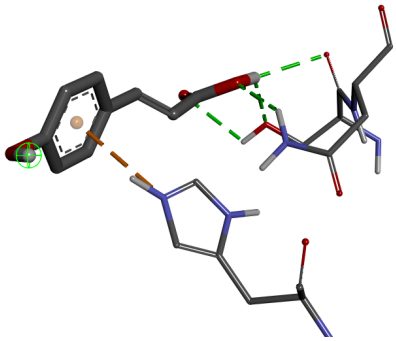


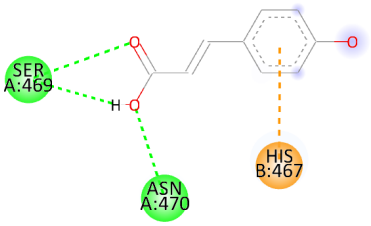
Ferulic acid
(Binding affinity:
-4.7 kcal/mol)



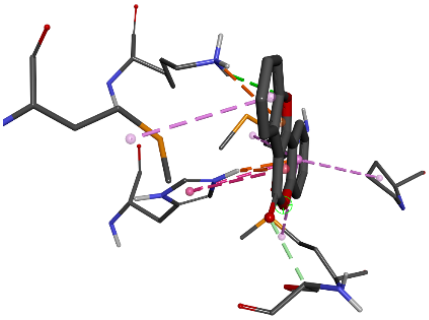


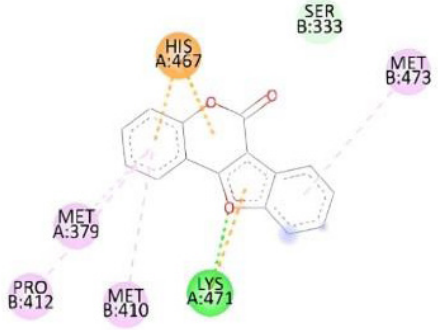
p-coumaric acid
(Binding affinity:
-4.5 kcal/mol)





Coumestan
(Binding affinity:
-5.2 kcal/mol)





The result of molecular docking reports that the isoflavone coumestan exhibits the strongest affinity and interaction with both the estrogen receptors α and β. In the case of estrogen receptor α, it exhibited a binding affinity of -8.0 kcal/mol, while in the case of estrogen receptor β, the binding affinity was found to be -5.2 kcal/mol. The binding affinities of the interaction of the apigenin, luteolin, genistein, daidzein, kaempferol, quercetin, myricetin, vanillic acid, caffeic acid, ferulic acid and p-coumaric acid with estrogen receptor α are -4.6 kcal/mol, -1.2 kcal/mol, -4.7 kcal/mol, -4.5 kcal/mol, -4.8 kcal/mol, -4.6 kcal/mol, -3.3 kcal/mol, -5.9 kcal/mol, -6.3 kcal/mol, -6.0 kcal/mol and -5.9 kcal/mol, respectively. The binding affinities of the interaction of the apigenin, luteolin, genistein, daidzein, kaempferol, quercetin, myricetin, vanillic acid, caffeic acid, ferulic acid and p-coumaric acid with estrogen receptor β are -4.0 kcal/mol, -4.7 kcal/mol, -3.5 kcal/mol, -3.7 kcal/mol, -4.0 kcal/mol, -5.2 kcal/mol, -3.4 kcal/mol, -5.0 kcal/mol, -4.9 kcal/mol, -4.7 kcal/mol and -4.5 kcal/mol, respectively. The interactions between the estrogen receptors and the phytochemicals are presented in Table 4.

The docking analysis also revealed that all three isoflavones present in black gram (genistein, daidzein, and coumestan) bind to the receptor at the same site, as they bind to the same set of amino acids. In the case of estrogen receptor α, the common amino acids that bind to the majority of the phytoestrogen includes GLU323, PRO324, GLU353, ARG394, and LYS449. Whereas, in case of estrogen receptor β MET410, HIS467, SER469, ASN470 and MET473 are the common amino acid residues binding to the different phytoestrogens present in functional idli. We also observed some minor unfavorable donor-donor binding at the GLU337, HIS475, TRP335 and ARG466, as well as ASN470 of estrogen receptor β with the phytoestrogens apigenin, quercetin, myricetin, and caffeic acid, respectively. Binding of vanillic acid with estrogen receptors α and β shows unfavorable acceptor-acceptor interaction with the GLU353 and ILE386 as well as GLU332 and SER333 residues, respectively, in addition to unfavorable donor-donor interaction with the residue HIS467. The impact of these unfavorable interactions, however, is minor resulting in efficient binding of the phytoestrogens with the receptors.

Table 4. Forces of interaction between the receptor and ligands

Receptor	Ligand	Conventional Hydrogen bonding	Pi anion	Pi alkyl and Alkyl	Pi cation	Carbon H bonding	Pi sulphur	Pi sigma	Pi-Pi stacked
Estrogen receptor α (PDB ID: 2YJA)	Apigenin	TRP393, LYS449	GLU323	PRO324, MET357	ARG394, LYS449	-	-	-	-
	Luteolin	GLU353, PHE404	-	LEU349, ALA350, LEU384, LEU387, MET388, LEU391, ILE424	-	-	-	MET388	-
	Genistein	LYS449, ILE386	GLU323, GLU353	PRO324, MET357	ARG396	GLY390	-	-	-
	Daidzein	LYS449	GLU232, GLU353	PRO324, LEU387	ARG394	GLY390	-	-	-
	Kaempferol	TRP393, LYS449	GLU323	PRO324	ARG394, LYS449	-	MET357	-	-
	Quercetin	GLU323, PRO325, TRP393, LYS449	GLU353	PRO324	ARG394	HIS356	-	-	PHE445
	Myricetin	GLU323	GLU353	PRO324	ARG394, LYS449	-	-	-	-
	Vanillic acid	ARG394	GLU353	PRO324	LYS449	GLY390	-	-	-
	Caffeic acid	LEU346, GLU353	-	ALA350, LEU387, LEU391	-	-	-	-	PHE404 (Pi-Pi T shaped)
	Ferulic acid	-	GLU353	PRO324, LEU327, MET357	-	PRO325, TRP393	-	-	-
	p-coumaric acid	LEU387	-	ALA350, LEU391	-	-	-	-	PHE404 (Pi-Pi T shaped)
	Coumestan	LYS449	GLU323	PRO324	ARG394	-	MET357	MET388	-
Estrogen receptor β (PDB ID: 5TOA)	Apigenin	MET336	-	MET473	HIS467, LYS471	-	-	MET379	-
	Luteolin	GLU332, SER333, MET410	-	MET473	HIS467, LYS471	-	MET410	-	-
	Genistein	MET410	-	MET379, PRO412, MET473	HIS467	-	-	-	-
	Diadzein	LYS471	-	MET379, PRO412, MET473	HIS467	-	MET410	-	-
	Kaempferol	MET336, SER469, GLU375	-	MET473	HIS467, LYS471	-	-	MET379	TRP335 (Pi-Pi T shaped)
	Quercetin	LEU339	-	LEU298, ALA301, LEU336, LEU340, LEU343, ILE376, LEU476	-	-	-	-	-
	Myricetin	SER333, TRP335, GLU375, MET410, LYS471	GLU332	MET379, MET473	HIS467	-	MET410	-	HIS467
	Vanillic acid	-	-	HIS467, MET473	-	HIS467, ASN470	MET473	SER333	-
	Caffeic acid	SER469	-	-	HIS467	-	-	-	-

Table 4. (cont.)

Receptor	Ligand	Conventional Hydrogen bonding	Pi anion	Pi alkyl and Alkyl	Pi cation	Carbon H bonding	Pi sulphur	Pi sigma	Pi-Pi stacked
Estrogen receptor β (PDB ID: 5TOA)	Ferulic acid	GLU332, MET336, GLU337, HIS467, SER469	-	HIS467	-	-	-	-	-
	p-coumaric acid	SER469, ASN470	-	-	HIS467	-	-	-	-
	Coumestan	LYS471	-	MET379, MET410, PRO412, MET473	HIS467	SER333	-	-	-

The study presents the design of a novel functional idli that is rich in phytochemicals that act as phytoestrogens and can effectively bind with estrogen receptors α and β. Therefore, the proposed functional food may be efficient in reduction of post-menopausal adverse effects.

6. Conclusion

Food engineering has greatly improved the food quality and nutritional profile to overcome the issues of food security, food quality and disease management. Functional foods provide favourable physiological benefits in reducing the risk of disease development. Idli is an Indian traditional food, with an enhanced nutrition profile due to fermentation and steam cooking. The major ingredients in idli are rice and black gram. The proposed functional idli is made up of soybean, black gram, rice, oats, spinach, moringa and fenugreek seeds. These ingredients have a rich nutritional profile and possess numerous beneficial properties that can improve the health status of post-menopausal women. These ingredients are rich source of phytoestrogens that are beneficial for post-menopausal women with reduced estrogen production. The molecular docking approach helps to validate the efficacy of the proposed functional idli in prevention of post-menopausal conditions. From the list of phytochemicals analyzed, coumestan has the strongest binding affinity with both the estrogen receptors α and β. Therefore, the phytochemicals discussed can be effective in reducing the adverse effects of post-menopause caused by reduced level of estrogen and the phytochemical-based interventions can prove to be efficacious in designing novel prophylactic approaches. Further analysis is required to confirm the efficacy of the proposed functional food.

Acknowledgement

The authors want to express their gratitude to Dr. G. Viswanathan, Chancellor, VIT University, Vellore, for his constant encouragement and support. The authors also acknowledge the valuable suggestions from the peer reviewers.

Conflict of interest

The authors declare no competing financial interest.

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