

**EFFECTS OF SELECTED NATURAL COAGULANTS ON THE YIELD AND QUALITY CHARACTERISTICS OF *Glycine max* AND *Vigna subterranea* MILK MIXTURE TOFU**Ndatsu Y.¹, Abubakar H. ¹, Aliyu O. A.¹, Yisa P. S. ², Usman B. H.¹¹Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria. ²Niger State College of Agriculture, Department of Pre-ND Sciences, PMB.11, Mokwa, Niger State.*Corresponding Author: yndatsu2011@gmail.com**ABSTRACT**

The effects of selected natural coagulants on the yield and quality Characteristics of *Glycine max* and *Vigna subterranea* Milk Mixture Tofu was investigated. The selected natural coagulants are *Citrus lemon*, roselle calyces (*Hibiscus sabdariffa*), *Tamarindus indica*, and influence water (steep water) from maize pap, while CaSO₄ was used as a control group. The pH and titratable acidity values of the prepared tofu were performed. Tofu yield, proximate composition, and sensory quality of tofu prepared by natural coagulants of plant origins were also conducted and compared with that of CaSO₄ coagulated tofu. Results showed that natural coagulants recorded lower values of pH (3.54-20.00) and increased values of percentage (%) acidity (0.12-1.20%) as compared to synthetic coagulant (21.03 and 0.09%), respectively. Likewise, lower levels of pH (6.77-37.45) and high values of percentage (%) acidity (0.18-0.35%) were observed in natural coagulated tofu than that of synthetic chemical coagulated tofu (40.42 and 0.12%), respectively. CICOT and STCOT recorded higher % of tofu yield (21.5% and 45.2%), proximate contents and panel overall acceptability (7.43% and 7.63%) as compared to others. In conclusion, *citrus lemon* extract and steep water coagulated tofu performed excellent in term of tofu yield with high quality and nutritional contents. Therefore, the consumption of tofu produced from natural coagulants, especially citrus lemon and steep water should be encouraged in the diets of vegetarians as source of protein.

Keywords: Natural coagulants; tofu; coagulation; *Glycine max*; *Vigna subterranea***INTRODUCTION**

Glycine max L. (Soybean) is a leguminous crop widely grown and eaten all over the world as grains and soy foods (tofu, soymilk, soy sauce, miso, and soy cheese). In addition, soy meal (a byproduct of soy oil extraction) is used to feed animals (Kim *et al.*, 2010). The grain contains 40% protein, 20% lipid, majorly made of unsaturated fatty acids (Oleic acid and linoleic acid), 30% carbohydrate in combination with fibre, vitamins and mineral elements (Lim, 2012; Salem *et al.*, 2018; Kyung *et al.*, 2020). More also, it contains other chemicals that have health benefits e.g. phenols, flavonoids, isoflavones, saponins, phytoesters and sphingolipid (Lee *et al.*, 2008; Gutierrez *et al.*, 2004). All these are reported to have protective effects against oxidative stress related diseases like liver dysfunctions, cancer, obesity, and cardiac diseases (Bellaloui, 2012; Kyung *et al.*, 2020). Soybean is the cheapest food resources with antioxidant, estrogenic, antidiabetic, antihypercholesterolemic, antiobesity, anticancer, antiviral, antihypertensive, hepatoprotective antiskin aging, and antihyperlipidemic potentials due to its high profile of bioactive compounds as compared with other legume and animal feed sources (Lim, 2012; El-Shemy, 2013; Salem *et al.*, 2018). Messina (2010) has reported that the pharmacological activities exhibited by soybean are due to high content of isoflavones.

Vigna subterranea (L), commonly known as Banbara groundnut is leguminous crop belonging to the family

of Fabaceae (Messina, 1999; Victoria and Claudine, 2014; Victoria *et al.*, 2021). It is locally called Kpa-Roro in Nigeria, and it seed contains 57-67% carbohydrates, 15-27% proteins, and <10% lipids, the high proteins and fiber content of the seed signify its nutritive value in human diet and ability to curtail malnutrition (Oyeyinka *et al.*, 2018). Swanevelder (1998) and Victoria *et al.* (2014) have reported that high biological activity of Banbara ground nut is attributed to its high content of secondary metabolites. Traditionally, the medicinal uses of Banbara ground nut have been sighted by Koné (2011). The high profiles of anthocyanins, proanthocyanidin, malvidin, and flavonoids in Banbara ground nut are attributed to its health benefits (Pale *et al.*, 1997; Onyilagha *et al.*, 2009; Victoria and Claudine, 2014). High content of oxalate, saponins, alkaloids, vitamin E, vitamin A, and niacin have been reported in Banbara ground nut (Victoria and Claudine, 2014). The antioxidant potential of Banbara ground nut is reported to be the presence of flavanols, flavonols and anthocyanins (Victoria *et al.*, 2021).

Tofu is a protein-based food product normally produced from vegetable which is popularly consumed in the Western countries due to its inexpensive, nutrients content, easy digestibility, and high texture profile analyzed (Kim *et al.*, 2008; Ndatsu *et al.*, 2013; Shafa'atu *et al.*, 2022). It is mostly produced from soymilk using various forms of coagulants like salts

(CaSO₄, CaCl₂, MgSO₄ and MgCl₂), acids (glucono-delta-lactone), and natural coagulants (proteinase of plant extracts, steep water produced from pap of maize) (Wang and Hesseltine, 1982; Ndatsu *et al.*, 2013). Tofu is popularly eaten among the less privilege Orientals because of its health benefits (Poysa and Woodrow, 2002; Yakubu and Amuzat, 2012). Traditionally, tofu production is done by soaking the seed of the vegetable for almost 8 hrs under room temperature, grinding of seed with water and the residual fiber removed from the slurry (Shafa'atu *et al.*, 2022). The extract (milk) obtained will then be heated at 100 °C, coagulated using a coagulant and the whey will be removed from the coagulated curd using filter cloth (Muslim cloth) (Yakubu and Amuzat, 2012; Shafa'atu *et al.*, 2022). The yield and quality of tofu produced will be depended of varieties and quality of vegetable, vegetable seed-to-water ratio (Shen *et al.*, 1991), conditions of processing, coagulants involved, stirring speed and moulding time and pressure (Fasoyiro, 2014). In this work, soya beans (*Glycine max*) and Bambara groundnut (*Vigna subterranea*) mixture was used to produce tofu using selected natural coagulants of plant origins. The use of synthetic coagulants are sometimes not household accessible for use, and their availability in the rural areas are also difficult. Their uses also cause some problems if the processes of making tofu are not properly handled. For instance, insufficient addition of CaSO₄ causes abnormal coagulation of soy protein, which makes the sieving problematic while excess application of it causes the tofu's texture hard and not palatable (Tsai *et al.*, 1981; Sanjay *et al.*, 2008). Previously, the use of natural coagulants of plant origins such as *Citrus lemon*, *Garcinia indica*, *Tamarindus indica*, *Averrhoa carambola*, *Averrhoa bilumbi*, *Phyllanthus distichus*, *Tamarindus indica flower extract*, *Passiflora edulis* (Sanjay *et al.*, 2008), steep water from domestically processed maize pap (Ndatsu *et al.*, 2013; Fasoyiro, 2014) and roselle calyces (*Hibiscus sabdariffa*) (Sanjay *et al.*, 2008; Shafa'atu *et al.*, 2022) for high yield and better texture have been reported. For these reasons, an attempt was made in this work to examine the effects of selected natural coagulants on the yield and quality characteristics of *Glycine max* and *Vigna subterranea* seeds tofu.

MATERIALS AND METHODS

Materials

Glycine max L. (Soybean seeds), TGX923-1E variety, *Vigna subterranea* (Bambara groundnut), and maize grains (variety, DMR-ESR-Y) were collected directly from seed store, Faculty of Agriculture, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria. The calcium sulphate (CaSO₄) used was industrial grade. The selected natural coagulants from

plant origins (*Citrus lemon*, roselle calyces (*Hibiscus sabdariffa*), and *Tamarindus indica*) were purchased from local market in Lapai local Government, Niger State, Nigeria. These materials were cleaned, thoroughly by picking out the debris, stones and washed using tap water.

Coagulant preparations

Briefly, about 0.2% of CaSO₄ suspension was prepared and labeled as CACO for the preparation of tofu (control group). For the natural coagulants, 10% concentration of each plant was prepared by soaking 25 g of each in 250 mL of distilled water for 10 mins, ground using mortar and pestle. The extract obtained from each sample was filtered through a Whatman paper No. 1 to have the final natural coagulants labeled as CICO, HICO, and TACO, respectively. The fermented maize coagulant (steep water) was prepared following the method of Fasoyiro (2014). Briefly, about 25 g of maize grains were soaked in 250 mL of distilled water for 70 hrs and water was changed two times during the period of soaking. The fermented maize grains were then blended using a blender (Nakai Model 462, Japan) in 250 mL of distilled water. The slurry obtained was filtered through a muslin cloth, and final through Whatman paper No, 1 to have the coagulant, labeled as STCO.

Coagulants Acidity Measurement

The filtrate of each coagulant was titrated against 0.1N Sodium hydroxide (NaOH) using phenolphthalein as indicator. The acidity of each coagulant calculated in percent anhydrous lactic acid and then adjusted to 2% of anhydrous lactic acid using distilled water (Sanjay *et al.*, 2008).

Tofu Preparation

Briefly, 200g of soybeans and Bambara groundnut were measured each, and soaked together in 5000 mL of distilled water at 0 °C temperature for 10 h to remove the outer hull. The dehulled soybeans and Bambara groundnut were ground in 4000 mL of distilled water to have the slurry. The slurry was then filtered using muslin cloth to obtain about 4000 mL soybean and Bambara groundnut milk, boiled at 100 °C for 5 min and allowed to cool to 60 °C. Then 300 mL of soy milk were measured into five (5) different beakers (500 mL) while 0.2% of CaSO₄ coagulants (control group) was added into first beaker containing 300 mL of soymilk. Into beaker 2, 3, 4, and 5, 10% of natural coagulants (CICO, HICO, TACO and STCO) were added into each beaker containing 300 mL of soymilk, respectively, stirred for 5 sec, milk allowed coagulating for 20 min at the temperature of 0 °C, and curd and whey were separated. The curds were packed into muslin cloth lined wooden mold, pressed using 20 kg weight for 40 min. The weights of fresh tofu obtained were taken and

the cut tofu cakes were kept in the fridge at 5 °C for further works.

Tofu Yield

The weight of each fresh tofu (g) obtained from coagulated 300 mL of soy and Bambara nut milk was expressed as % yield (Fasoyiro, 2014).

Proximate Composition

The gravimetric method was used to evaluate the moisture content of each coagulated tofu (AOAC, 2000). The content of protein of each tofu was determined using the method of Kjeldahl, the factor of conversion was 6.25 (AOAC, 2000). The soxhlet extraction method was used for fat content determination and the ash, fibre, and carbohydrate contents were determined by the method of AOAC (2000).

Sensory Evaluation

Each sample of fresh tofu was coded and distributed among the twenty six (26) semi trained panel members that are familiar with quality and characteristic of tofu. The panel members were given the samples of tofu of the size as 3 cm x 3 cm x 3 cm on the plate and the assessment was based on the colour, appearance, flavour, firmness and over all acceptability following nine (9) point Hedonic scale (1: extremely dislike, 2: very much dislike, 3: moderately dislike, 4: slightly dislike, 5: like or dislike, 6: slightly like, 7: moderately like, 8: very much like, 9: extremely like) (Shafa'atu *et al.*, 2022).

Statistical Analysis

Triplicate analysis was performed, and results were expressed as mean \pm standard deviation. One-way analysis of variance and least significant difference (LSD) test were used to evaluate the significant difference. Probability levels of less than 0.05 were considered significant.

RESULTS

The results of pH and percentage acidity of coagulants used are shown in Table 1. The pH and the percentage (%) titratable acidity values of coagulants varied from 3.54–21.03 and 0.09% - 1.21%, respectively. Chemical coagulant (CACO) recorded high significant value of pH than those values in natural coagulants. Among the natural coagulants, CICO recorded high value of pH than others, however natural coagulants recorded high values of percentage (%) acidity than that of the chemical coagulant (CACO). Among the natural coagulants, CICO and STCO had high significant values of pH (20.00) and percentage (%) acidity (1.20%), respectively than others. The high trend of

percentage acidity recorded in the coagulants are arranged as steep water>*Tamarindus indica*>*Hibiscus sabdariffa*>*Citrus lemon*>CaSO₄ (Table 1).

Table 1: pH and titrated acidity of the coagulant filtrates

Coagulant	Conc. (%)	pH	Titrated acidity (%)
CACO	10	21.03 \pm 0.01 ^a	0.09 \pm 0.01 ^e
CICO	10	20.00 \pm 0.02 ^b	0.12 \pm 0.01 ^d
HICO	10	4.59 \pm 0.02 ^c	0.51 \pm 0.02 ^c
TACO	10	5.01 \pm 0.01 ^c	0.94 \pm 0.03 ^b
STCO	10	3.54 \pm 0.01 ^d	1.20 \pm 0.02 ^a

Means with the same superscript within same column are insignificantly different ($p>0.05$). CACO: CaSO₄ coagulant; CICO: *Citrus lemon* coagulant extract; HICO: *Hibiscus sabdariffa* coagulant extract; TACO: *Tamarindus indica* coagulant extract; STCO: Steep water coagulant extract.

The effect of coagulants on the pH values and the titratable percentage (%) acidity of coagulated tofus are presented in Table 2. It reveals that the pH values and the percentage acidity varied from 6.57 to 40.42 and 0.12 to 0.34% respectively. Decreased significant levels of pH were recorded in natural coagulated tofu than that of chemical coagulated tofu (CACOT). However, high values of percentage (%) acidity were recorded in natural coagulated tofu than that of the CACOT. The ranges of pH values and % acidity of the coagulated tofu are arranged in the following trend: CACOT>CICOT>TACOT>HICOT>STCOT and STCOT>TACOT>HICOT>CICOT>CACOT, respectively (Table 2).

Table 2: pH and titrated acidity of coagulated tofu

Tofu samples	pH	Titrated acidity (%)
CACOT	40.42 \pm 0.01 ^a	0.12 \pm 0.02 ^d
CICOT	37.45 \pm 0.01 ^b	0.18 \pm 0.02 ^c
HICOT	7.15 \pm 0.03 ^d	0.21 \pm 0.03 ^b
TACOT	10.23 \pm 0.03 ^c	0.25 \pm 0.01 ^b
STCOT	6.77 \pm 0.01 ^d	0.34 \pm 0.01 ^a

Means with the same superscript within same column are insignificantly different ($p>0.05$). CACOT: CaSO₄ coagulated tofu; CICOT: *Citrus lemon* coagulated tofu; HICOT: *Hibiscus sabdariffa* coagulated tofu; TACOT: *Tamarindus indica* coagulated tofu; STCOT: Steep water coagulated tofu.

The effect of coagulants on the yield and proximate analysis of tofu are shown (Table 3). All the tofu coagulated by natural coagulants had increased significantly at $p>0.05$ of yield and proximate

compositions analyzed than the control, chemical coagulant (CACOT). Tofu yields varied from 17.3 to 45.2 %, which was arranged in the following trend: STCOT>CICOT>TACOT> HICOT>CACOT (Table 3). STCOT and CICOT have the highest moisture contents (54.3, 53.8 %) respectively, which are not differed significantly as compared to others. Similarly, higher and not significant differs ($p>0.05$) of protein contents were recorded in CICOT (39.2 %) and STCOT

(38.9%) than the others. In addition, high fat levels were observed in CICOT (23.5%), and TACOT (23.2%), which are not differed significantly at $p>0.05$ from each other. Insignificant higher values at $p>0.05$ of ash were observed in STCOT (6.4%), CICOT (6.3%), HICOT (6.2%), and TACOT (5.9%) than other groups. STCOT shows increased significant level of fibre and carbohydrate contents (20 and 55.5%), respectively than others (Table 3).

Table 3: Yield and proximate compositions of coagulated tofu

Tofu samples	Yield	Moisture	Protein	Fat	Ash	Fibre	Carbohydrate
Percentage (%)							
CACOT	17.5±0.12 ^d	42.3±0.21 ^c	31.2±0.30 ^d	13.4±0.10 ^d	4.4±0.01 ^c	1.3±0.02 ^d	22.2±0.10 ^d
CICOT	21.5±0.21 ^b	53.8±0.12 ^a	39.2±0.14 ^a	23.5±0.21 ^a	6.3±0.01 ^a	1.6±0.02 ^b	45.8±0.12 ^b
HICOT	19.3±0.10 ^c	52.0±0.11 ^b	33.5±0.20 ^c	21.0±0.22 ^b	6.2±0.02 ^a	1.5±0.01 ^c	29.4±0.03 ^c
TACOT	21.1±0.11 ^b	52.4±0.13 ^b	35.9±0.21 ^b	23.2±0.12 ^a	5.9±0.01 ^a	1.5±0.02 ^c	45.5±0.20 ^b
STCOT	45.2±0.22 ^a	54.3±0.23 ^a	38.0±0.21 ^a	15.5±0.13 ^c	6.4±0.01 ^a	2.0±0.03 ^a	55.5±0.21 ^a

Means with the same superscript within same column are insignificantly different ($p>0.05$). CACOT: CaSO₄ coagulated tofu; CICOT: *Citrus lemon* coagulated tofu; HICOT: *Hibiscus sabdariffa* coagulated tofu; TACOT: *Tamarindus indica* coagulated tofu; STCOT: Steep water coagulated tofu.

Sensory scores evaluation

The results for panel of tofu tasted for sensory scores are presented in the Table 4. All tofu coagulated using natural coagulant extracts had significantly increase of sensory scores as compared to that of tofu coagulated using chemical coagulant (CACOT). The coagulated tofu, STCOT and CICOT were rated to have comparably high sensory scores for appearance, firmness, and overall acceptability as compared to others. In addition, high significant values at $p>0.05$ of colour and flavour were recorded in HICOT and CICOT coagulated tofu, respectively, as compared to others (Table 4).

Table 4: Mean sensory evaluated of coagulated tofu

Tofu samples	Appearance	Colour	Flavour	Firmness	Overall acceptability
CACOT	3.81±0.12 ^c	3.71±0.10 ^c	4.50±0.13 ^d	3.00±0.12 ^c	4.25±0.02 ^c
CICOT	7.20±0.14 ^a	4.17±0.12 ^b	7.21±0.11 ^a	6.82±0.13 ^a	7.43±0.01 ^a
HICOT	6.72±0.11 ^b	6.02±0.13 ^a	5.43±0.12 ^c	6.51±0.11 ^b	6.12±0.10 ^b
TACOT	6.54±0.12 ^b	5.84±0.13 ^a	6.02±0.11 ^b	6.64±0.21 ^b	6.74±0.03 ^b
STCOT	7.25±0.13 ^a	4.01±0.10 ^b	5.34±0.14 ^c	7.14±0.12 ^a	7.63±0.02 ^a

Means with the same superscript within same column are insignificantly different ($p>0.05$). CACOT: CaSO₄ coagulated tofu; CICOT: *Citrus lemon* coagulated tofu; HICOT: *Hibiscus sabdariffa* coagulated tofu; TACOT: *Tamarindus indica* coagulated tofu; STCOT: Steep water coagulated tofu.

DISCUSSIONS

The variations of pH values and % acidity obtained in different coagulants and coagulated tofu signifies their acidic nature. The pH values and % acidity variation in various coagulants and coagulated tofu have been reported that pH and % acidity of tofu is dependent on the coagulant type (Sanjay et al., 2008; Fasoyiro, 2014). Shafa'atu et al. (2022) have reported the variation of pH values and percentage (%) acidity in various concentrations of roselle extracts and the prepared tofu.

The variations in % acidity of the prepared tofu depend on the nature of coagulant (Fasoyiro, 2014). For tofu coagulation to take place, pH is considered as one of the major factors. However, pH has been reported to be invaluable in term of tofu yield (Cao et al., 2017). The high yield of tofu coagulated using natural coagulant extracts than that of chemical coagulant may be attributed to the coagulant types and not the pH level of the coagulants. This could be an indication that natural coagulants ability for tofu coagulation is more

than that chemical coagulant. Ndatsu and Olalekan, (2012) have demonstrated the ability of various coagulants for tofu coagulation. Higher tofu yield by natural coagulants than that of chemical coagulant could be due to addition of extraneous substances from the natural coagulants to tofu. Addition of extraneous substances to tofu yield from coagulants has been reported (Yakubu et al., 2012). It has been reported that for tofu coagulation to be effected, coagulant types is one of the major factors rather than the pH values of the coagulants (Cao et al., 2017). The coagulant types had been reported to have positive effects on tofu yields (Shafa'atu et al., 2022). Least production of tofu coagulated using CaSO_4 as a coagulant than coagulant from plant origin had been reported (Obatolu, 2008; Prabhakaran et al., 2006; Shafa'atu et al., 2022). A report on the variation of tofu yield using various coagulants had been demonstrated (Obatolu, 2008). In addition, higher protein levels observed in the natural coagulated tofu than that of CaSO_4 coagulated tofu may signify their higher protein content and why proteins retention while pressing. This increased in proteins recovery in the plant extracts coagulated tofu may be probably due to the possibility of proteins in the extracts been taking over to their coagulated tofu. Ndatsu and Olalekan, (2012) have narrated that the movement of protein contents from steep water into its coagulated tofu contributed to the higher levels protein in the steep water coagulated tofu. The ionic strength differences of coagulants do affect their capacity of coagulations and quality of extracted milk-protein being coagulated and subsequently, affect the protein levels of the tofu produced (Ndatsu and Olalekan, 2012). Sanjay et al. (2008) have demonstrated that the processes of tofu coagulation and pressing can cause the carbohydrate removal and increased in protein contents on the basis of dry matters. The difference in the protein contents could be a reflection of coagulant effects on the glysin and β -conglysinin cross-linkages (Sanjay et al., 2008). Higher proteins recovery has been reported in tofu coagulated by plant extracts (Sanjay et al., 2008). Fasoyiro, (2014) has reported the high recovery of protein levels in roselle extract coagulated tofu. Sanjay et al. (2008) have demonstrated that the processes of tofu coagulation and pressing can cause the carbohydrate removal and increased in protein contents on the basis of dry matters. The difference in the protein contents could be a reflection of coagulant effects on the glysin and β -conglysinin cross-linkages (Sanjay et al., 2008). Higher proteins recovery has been reported in tofu coagulated by plant extracts (Sanjay et al., 2008). Fasoyiro, (2014) has reported the high recovery of protein levels in roselle extract coagulated tofu. Furthermore, increased values of fats recorded in plant extract tofu than that of control group could be attributed to the high coagulated protein in the milk that

might have absorbed lipid into the matrix of tofu. Ndatsu and Olalekan (2012) have reported that tofu matrix tripped the available fats due to higher content of coagulated protein in the milk. In contrast, lower lipid values of tofu may be accounted for higher tofu yields. Shafa'atu et al., (2022) have narrated higher lipid contents can facilitate free coagulation processes to give high yield of tofu than the tofu with lower lipid content due to higher interactions between protein-protein and protein-water over the lower lipid content. Similarly, higher moisture values of plant extract coagulated tofu as compared to the control group might signify their higher water retention capacity, which might be due to free interaction of protein with water in the matrix of the tofu. This might also be attributed to their higher yields compared to the control group (Shafa'atu et al., 2022). The ash contents (4.4-6.4g/100g) of various tofu reported in this work is generally higher than what was demonstrated by Fasoyiro, (2014) and Shafa'atu et al. (2022) as 4.1-6.0g/100g and 0.85-2.00, respectively, which could be attributed to differences in tofu making procedures and the samples used in making tofu. Increased values of proximate indices noted in various tofu produced signify the effects of coagulants used in tofu preparation (Ndatsu and Olalekan, 2012). Both fibre and carbohydrate contents in all tofu coagulated using natural coagulants are comparatively higher than that of control group.

More again, overall acceptability of tofu coagulated by plant extracts than that of CaSO_4 coagulated tofu are reflection of their quality characteristics accepted by panel in term of appearance, colour, flavour and firmness. Higher acceptance scores received by CICOT and STCOT than other groups may be attributed to their high rate of panel scores in term of appearance, flavour and firmness. Higher acceptability of tofu prepared by citrus lemon (CICOT) and steep water (STCOT) has been reported (Sanjay et al., 2008). Lower acceptance of panel to tofu prepared by CaSO_4 coagulated tofu (Shafa'atu et al., 2022).

CONCLUSION AND RECOMMENDATION

This study shows the possibility of producing tofu using plant extracts as coagulants instead of synthetic chemical coagulants. It was also revealed that citrus lemon and steep water extracts performed excellent in term of tofu yield with high quality and nutritional contents. Therefore, tofu produced from natural coagulant should be recommended to vegetarians as an alternative source of protein.

Further works on total antioxidant and hepatoprotective activity of soybean and Banbara groundnut milk mixture tofu prepared using natural coagulants is recommended.

REFERENCES

- AOAC (2000). Official Methods of Analysis, 16th edition. Association of Official Analytical Chemists, Washington, DC
- Bellaloui, N. (2012). Soybean Seed Phenol, Lignin, and Isoflavones and Sugars Composition Altered by Foliar Boron Application in Soybean under Water Stress. *Food Nutr. Sci.* 3:579–590.
- Cao, F. H., Li, X. J., Luo, S. Z., Mu, D. D., Zhong, X. Y., Jiang, S.T., Zheng, Z., and Zhao, Y. Y. (2017). Effects of Organic Acid Coagulants on the Physical Properties of and Chemical Interactions in Tofu. *LWT - Food Sci Technol.*, 85, 58–65.
- DeMan, J.M., DeMan, L., and Gupta, S. (1986). Texture And Microstructure of Soybean Curd (Tofu) as Affected by Different Coagulants, *Food Struct.*, 5(1), 11.
- El-Shemy, H. (2013). Soybean. Nutrition and health. In: El-Shemy, editor. Soybean-BioActive Compounds. Rijeka: *InTech*; 453-473
- Fasoyiro, S.B (2014). Physical, Chemical and Sensory Qualities of Roselle Water Extract-coagulated Tofu Compared with Tofu from Two Natural Coagulants. *Nigerian Food Journal*, 32(2); 97-102.
- Gutierrez, E, Wang, T, Fehr, W.R. (2004). Quantification of sphingolipids in soybeans. *Journal of the American Oil Chemists' Society.* 81:737-742
- Kim, W.S., Ho, H.J., Nelson, R.L., and Krishnan, H.B. (2008). Identification of Several Gy4 Nulls From the USDA Soybean Germplasm Collection Provides New Genetic Resources for the Development of High-Quality Tofu Cultivars, *J. Agric. Food Chem.*, 56(23), pp.11320–11326.
- Kim, H.G., Kim, G.W., Oh, H., Yoo, S.Y., Kim, Y.O., Oh, M.S. (2010). Influence of roasting on the antioxidant activity of small black soybean (*Glycine max* L. Merrill) *LWT Food Sci. Technol.* 2011;44:992–998. doi: 10.1016/j.lwt.2010.12.011. [[CrossRef](#)] [[Google Scholar](#)]
- Koné. M., Paice, A.G. and Touré, Y. (2011). Bambara groundnut [*Vigna subterranea* (L.) Verdc. (Fabaceae)] usage in human health. In: *Nuts and Seeds in Health and Disease Prevention* (edited by V.R. Preedy, R.R. Watson & V.B. Patel). UK: Elsevier Inc.; 192-194
- Kyung, J. Lee, D.B., Gi-An, L., Gyu-Taek, C., Yoon-Sup, S.O., Jung-Ro Lee, K.M., Jong-Wook, C. and Do, Y. (2020). Phytochemical and Antioxidant Activity of Korean Black Soybean (*Glycine max* L.) Landraces. *Antioxidants (Basel)*. 9(3): 213.
- Lee, S.J, Kim, J.J, Moon, H.I, Ahn, J.K, Chun, S.C, Jung, W.S, Lee, O.K, Chung, I.M. (2008). Analysis of isoflavones and phenolic compounds in Korean soybean *Glycine max* (L.) seeds of different seed weights. *Journal of Agricultural and Food Chemistry.* 56:2751-2758
- Lim, T.K, (2012). Edible Medicinal and Non-Medicinal Plants. Vol. 2, Fruits. New York: Springer Science, Business Media B.V; 2012
- Messina, M. J. (1999). Legumes and soybeans: Overview of their nutritional profiles and health effects. *Am. J. Clin. Nutr.* 70, 439S-450S.
- Messina, M. (2010). Soybean isoflavone exposure does not have feminizing effects on men, a critical examination of the clinical evidence. *Fertility and Sterility.* 93:2095-2104
- Ndatsu Y., Ganiyu O., and Amuzat A. O. (2013). Antioxidant and Hepatoprotective Properties of Tofu (*Curdle Soymilk*) against Acetaminophen-Induced Liver Damage in Rats. *Biotechnology Research International*, 2013, 7 pages
- Onyilagha, J.C., Islam, S. and Ntamatungiro, S. (2009). Comparative phytochemistry of eleven species of *Vigna* (Fabaceae). *Biochemical Systematics and Ecology*; 37 16-19.
- Oyeyinka, S. A. et al. (2018). Value added snacks produced from Bambara groundnut (*Vigna subterranea*) paste or flour. *LWT-Food Sci. Technol.* 88, 126–131.
- Pale, E., Nacro, M., Vanhaelen, M. and Vanhaelen F. (1997). Anthocyanins from Bambara Groundnut (*Vigna subterranea*). *Journal of*

- Agricultural and Food Chemistry*; 45 3359-3361.
- Prabhakaran, V. A. (2008). Effect of Different Coagulants on Yield and Quality of Tofu from Soymilk. *Eur. Food Res. Technol.*, 226(3), 467–472.
- Prabhakaran, M. P., Perera, C. O., and Valiyaveetil, S. (2006). Effect of Different Coagulants on the Isoflavone Levels and Physical Properties of Prepared Firm Tofu. *Food Chem.*, 99(3), 492–499.
- Poysa, V. and Woodrow, L., (2002). Stability of soybean seed composition and its effect on soymilk and tofu yield and quality,” *Food Research International*, vol. 35, no. 4, pp. 337–345, 2002.
- Sanjay, K R., Subramanian, R., Senthil, Amudha and Vijayalakshmi, G. (2008). Use of Natural Coagulants of Plant Origin in Production of Soycurd (Tofu). *International Journal of Food Engineering*, 4(1); 1-13.
- Shafa'atu, G.I., Masud, E.S., Fatimah, D.K., Amina, R.M., Fatimah, J.I. (2022). Effect of Coagulants on Yield and Quality Characteristics of *Hibiscus cannabinus* Seed Tofu. *International Journal of Agriculture and Food Science Technology*, 13(1), 1-12
- Salem, A., Hussein, M., Muhammad K., Ehab H.E., Megahed, A., Muhammad, F. and Muhammad, A. (2018). Phytochemical Profiling of Soybean (*Glycine max* (L.) Merr.) Genotypes Using GC-MS Analysis. A book chapter: Phytochemicals - Source of Antioxidants and Role in Disease Prevention. *Intechopen*, 140 - 159
- Shen, C.F., De Man, L., Buzzell, R. I., and Man, J. D. (1991). Yield and Quality of Tofu as Affected by Soybean and Soymilk Characteristics: Glucono-Delta-Lactone Coagulant. *J. Food Sci*, 56(1), 109–12
- Swanevelder, C.J. (1998). Banbara—Food for Africa (*Vigna subterranea* – Banbara groundnut). Pp. 1-16. South Africa: National Department of Agriculture; 1998. p1-16.
- Tsai, S. J., Lan, C.Y., Kao, C. S., and Chen, S. C. (1981). Studies on the yield and quality characteristic of Tofu. *Journal of Food Science*. 46:1734-1737.
- Victoria, A., J. and Claudine, F. D. (2014). Nutritional, Therapeutic, and Prophylactic Properties of *Vigna subterranea* and *Moringa oleifera*. A book chapter on Antioxidant-Antidiabetic Agents and Human Health. *Intechopen*, 188-207
- Victoria, A. J., Bukola, C.A., Tonna, A.A., Malcolm, J.C.T., Fanie, R., Marilize, L.H. (2021). Phytochemical composition and antioxidant properties of methanolic extracts of whole and dehulled Banbara groundnut (*Vigna subterranea*) seeds. *Nature Scientific Reports*, 1-11
- Wang, H.L, and Hesseltine, C.W. (1982). Coagulation conditions in tofu processing. *Process Biochemistry* 17: 7 – 11.
- Yakubu, N. and Amuzat, A.O. (2012). Effects of Different Types of Coagulants on the Nutritional Quality Tofu Produced in the Northern Part of Nigeria. *World Journal of Dairy & Food Sciences*, 7 (2); 135-141.