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Quality Assessment of Tofu from Black Soybean (*Glycine max*.) by using Natural and Synthetic Coagulant

Susmita Adhikari[1] and Tika B. Karki[2*]

[1]National College of Food Science and Technology, Khusibu, Kathmandu, Nepal

[2]Department of Biotechnology, Kathmandu University, Dhulikhel, Nepal

Abstract

The quality assessment of different types tofu (normal, silken and intermediate dried) prepared from black soybean (*Glycine max*) using lime juice and calcium chloride in terms of yield, proximate composition, sensory analysis and functional properties. Curds were prepared using 2.5% of calcium chloride and 2.5% lime juice respectively based on soymilk (acidity as 6.88% citric acid). The lime juice produced significantly ($p<0.05$) higher yield percentage compared to calcium chloride on dry basis respectively. The proximate analysis revealed that the protein content, fat, ash and carbohydrate differed significantly ($p<0.05$). The lemon juice coagulated tofu had the highest protein content but lowest carbohydrate content in intermediate dried tofu on dry basis. The phytic acid content was found to be decrease in all types of tofu, whereas oxalate content increased in all tofu prepared using lemon juice. Silken tofu coagulated by calcium content showed higher total phenolic content compared to that of other tofu samples. Similarly, silken tofu coagulated with calcium chloride showed higher radical scavenging activity compared to other types of tofu coagulated by lemon juice and calcium chloride. From the sensory evaluation, normal tofu coagulated by lemon juice was better preferred compared to that of other different types of tofu.

Keywords: Natural coagulant, synthetic coagulant, Antioxidant, Total phenolic content, Anti-nutritional factor

Introduction

Tofu, also known as soybean curd, is a soft cheese- like food made by curdling fresh soy milk with coagulant. Traditionally, it is produced by curdling fresh hot soymilk with either salt ($CaCl_2$ or $CaSO_4$) or an acid. The coagulant produces a soy protein gel, which traps water, soy lipids and other constituents in the matrix forming curds. The curds are then pressed into solids cubes. The coagulation of soymilk relies on the complex inter relationship between type of soybean, soymilk cooking temperature, volume, solid content, pH, coagulant type, amount and time. However, there is a paucity of information on the study in black soybean tofu nor on the effect of natural coagulant (Lemon juice) on the variety of tofu (Hesseltine and Wang, 1982). Further, lemon juice is readily available in the local market in all seasons which could help small- scale processors in tofu preparation. This study was therefore carried out to determine the effect of natural coagulant on variety of tofu and compare it with the synthetic coagulant i.e. CaCl2. The maximum condition of coagulation process is indicated by appearing of almost transparent whey. Temperature also plays a role in coagulation process. The coagulating tofu at high temperature will increase the firmness of tofu but reduces the bulk yield. Among a variety of soybeans, the black soybean is reported to have discriminating components, such as phenolic acids, anthocyanins and isoflavones (Xu and Chand, 2008) and is also known to display superior biological activities to yellow and green soybeans, such as free radical scavenging activities and inhibition of LDL oxidation (Astadi *et al.,* 2009; Takahashi *et al.,* 2005; Xu and Chang, 2008) and anticancer (Hung *et al.*, 2007) activities of black soybean have also been reported. On the other hand, lemon juice as a coagulant could serve as the cheap source as compared to expensive salts (Gartaula *et al.,* 2013).

Several studies have focused on better selection of synthetic coagulants relating to improved quality and yield of tofu. However, research on the use and application of natural coagulant is still lacking. In addition, the effect of natural coagulant on different varieties of tofu (normal, silken and intermediate dried) is yet to be explored. This investigation will provide the basic knowledge in respect of exploring soy- based tofu development by natural coagulant replacing and/or substituting chemical coagulant in the perspective of

Nepal. This investigation believed to uplift the market and exposure to new product with small investment which simply benefit both the consumers and producers.

Materials and Methods

Materials

Common black variety of soybean named *Glycine max* was collected from the local market near the NIST complex Naybazar, Kathmandu, Nepal, and stored in the polyethylene bag. The coagulant calcium chloride dehydrate were of Fisher Scientific Inc., while lemon was brought from local market of Asan, Kathmandu, Nepal.

Preparation of normal tofu

200gm of black soybean was taken separately for each of the tofu variety and were soaked for 12 hours separately. The swelled beans were dehulled manually and ground by means of grinder. Grinding was done with intermittent addition of water. The bean and water ratio were maintained at 1:10 ratio by addition of water. The mash was heated and boiled for 10 mins and filtered through muslin cloth.

The temperature of milk was lowered to 75°C. then the coagulant i.e., lemon juice and calcium chloride (2.5%) was added respectively as described above with continuous stirring until the addition was complete. The curd was transferred to the pressing box lined with muslin cloth.

Preparation of silken tofu

For the preparation of soymilk, the procedure was repeated as for the normal tofu.

The temperature of milk was brought to 98°C. Then the coagulant as described above was added with continuous stirring until the addition was complete. The curd was transferred to the sealing container followed by indirect heating at 85°C and stored at 4°C.

Preparation of Intermediate dried tofu

For the preparation of soymilk and intermediate dried tofu same process was repeated as for the normal tofu.

The prepared normal tofu was dried at 55°C.

Chemical analysis

All raw materials and processed products were immediately analysed in laboratory.

Proximate Analysis:

Proximate composition (moisture content, crude fat, crude protein, crude fibre, crude ash) of different types of tofu was determined as per AOAC, 2005.

Percentage of yield of tofu

Percentage yield of tofu was calculated as per Yield of tofu solid (%) = $\frac{\text{Wt.of dry matter in tofu (g)} \times 100}{Wt.of\ dry\ matter\ in\ soyabean\ (g)}$

Anti-nutritional factors

Oxalate content was determined as per Patail and Gaidwad (1968). Oxalate content was determined as per Patail and Gaidwad (1968). 1g oven dried sample along with 10ml 3N HCl and 65ml distilled water was taken in 100ml volumetric flask and boiled for 1hr. after cooling the sample was diluted to 100ml and filtered. Two aliquots of 50 ml extract with 20ml 6N HCl were evaporated to half and filtered by washing several times. To filtrate 3-4 drops of methyl red indicator was added and to this concentrated ammonia solution was added until the solution turned faint yellow. The solution was heated on water bath 90-100°C temperature and to this 10ml 5% $CaCl_2$ was immediately added with 20-25 drops of ammonia solution to restore yellow color. The solution after incubating overnight was filtered through ash less filter paper and washed several times. Along with residue the filter paper was dissolved in hot 1:5 H_2SO_4 and diluted to 125ml. After heating to 90-100°C temperature, it was titrated against 0.05N $KMnO_4$. Percent oxalate was calculated by using formula:

% Oxalate = ml KMnO4 used × 0.05×45.02× 10/ 1000×dry weight×0.5

Phytic acid was determined by the method described by Sadasivam and Manickam (2008) using the formula:

Phytate, P mg/100g sample = µg Fe in sample ×15 / weight of sample (g)

Total phenol Preparation of Extract

The extract was prepared by cold percolation method according to Patel (2016).

Determination of Total phenols

Total phenols were determined with the Folin-Ciocalteau reagent (Makkar *et al.,* 1993). Half of a ml methanol solution of the concentrated solution will be mixed with 2.5 ml of 10% F-C reagent, and 5 minutes later, 2.5 ml of sodium carbonate (7.5% w/v) were added Then the mixed sample was incubated in an incubator at 45° for 45 minutes. The absorbance was measured at 765nm against a reagent blank. A standard calibration plot was generated using known concentration of Gallic acid (GA). The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg of Gallic acid equivalent (GAE) of phenol/ 100g of dry sample.

Antioxidant activity

Determination of DPPH radical scavenging activity

Determination of Antioxidant activity by DPPH radical scavenging method was according to the method described by Brand- William *et al.* (1995). Different concentration of 2 ml concentrated extract (12.5, 25, 50, 100, 200, and 400 µl/mg in methanol) was added to 3ml of 0.004% (w/v) DPPH solution. After 30 minutes incubation in darkness at room temperature, the reaction of the DPPH free radical was measured by reading the absorbance at 517 nm. DPPH is purple- coloured stable free radical; when reduced it becomes yellow- coloured diphenyl-picrylhydrazine. The corresponding percentage of inhibition was then calculated by using the formula:

Standard solution

Analytical grade ascorbic acid solution simply containing all the reagents in place of extracts was used. Ascorbic acid solution of 400µg/ ml, 200µg/ ml, 100µg/ ml, 50µg/ ml, 25µg/ ml and 12.5 µg/ ml were prepared.

Sensory evaluation

Sensory evaluation of tofu prepared was performed for aroma, color, texture, and appearance. For the sensory evaluation, ten semi-trained panellists among the students and staff of National College of Food Science and Technology, Kathmandu, Nepal were participated. Composite Scoring test as suggested by Ranganna was adopted as a method of analysis.

Statistical data analysis

Data were analysed in triplicate and subjected to the analysis of variance (ANOVA) using the Statistical Analysis System using the Genstat program. Significant levels were defined as probabilities of 0.05 or 5 % level of significant. The sample means were compared at

95% level of significance-test by using SPSS and Microsoft Office Excel 2015 and the best treatment was selected.

Results and Discussion Anti- nutritional Factor

Oxalate and Phytic acid content of different tofu prepared by lemon juice and calcium chloride was determined.

Oxalate: There was a significant difference ($p<0.05$) between the tofu's prepared by lemon juice and Calcium chloride. The oxalate content of normal tofu was found to be highest among different types of tofu in both coagulants (**Table 1**).

Phytate: The phytates content of normal tofu was found to be highest as compared to silken and intermediate dried tofu. There was a no significance difference ($p>0.05$) between T3, T4, T5 and T6. (**Table 1**).

Karki *et al.,* (2014) reported that phytic acid and oxalate content in tofu decreased with increase in coagulation temperature.

Table 1. Changes in anti-nutritional factors of normal, silken and intermediate dried tofu

Tofu	% Oxalate	Phytates g/100g
T1	0.41±0.01	1.31±0.02
T2	0.35±0.01	1.27±0.02
T3	0.06±0.01	0.22±0.01
T4	0.03±0.02	0.12±0.01
T5	0.32±0.01	0.31±0.01
T6	0.25±0.01	0.28±0.01

*Data are expressed as mean ± standard deviation

**T1: Lemon juice coagulated Normal tofu;

T2: Calcium chloride coagulated Normal tofu

**T3: Lemon juice coagulated Silken tofu;

T4: Calcium Chloride coagulated Silken tofu

** T5: Lemon juice coagulated intermediate tofu;

T6: Calcium chloride coagulated intermediate dried tofu.

Total Phenolic content

Phenolic content of T1, T2, T3, T4, T5 and T6 was found to be 262.77±4.39, 235.96±5.65, 212.02±4.01, 281.5±6.63, 151.78±4.70 and 206.3±6.95 mg GAE/100 gm respectively by using Gallic acid standard curve (R^2=0.9987). From the statistical analysis (ANOVA), $p<0.05$ indicated there was a significant difference between the phenolic contentamong all of the sample.

Normal tofu coagulated by using calcium chloride had the highest phenolic content (281.5±4.0) whereas intermediate dried tofu coagulated by using calcium chloride had lowest phenolic content (151.78±4.70) (**Figure 2**).

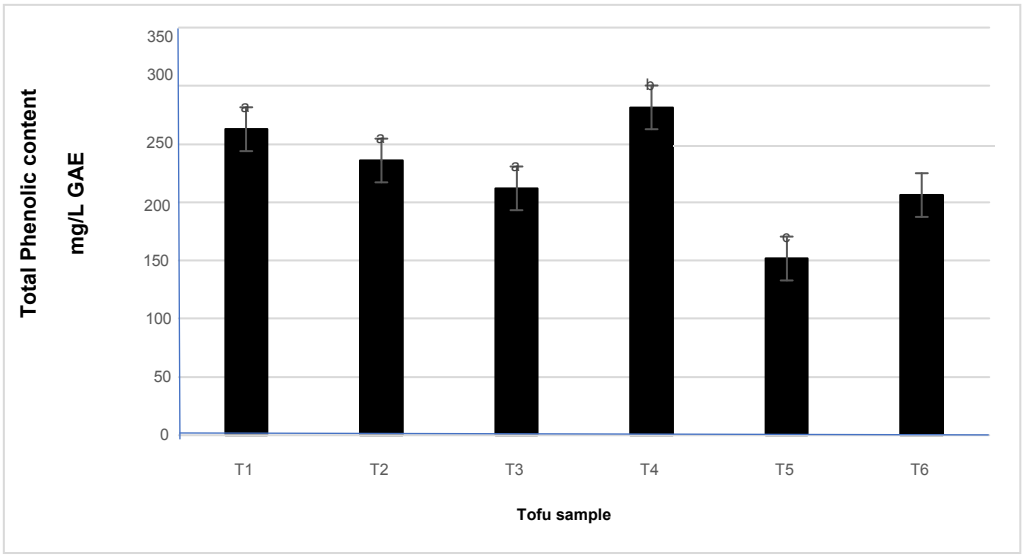

Fig 2. Total Phenolic content of Tofu sample

Antioxidant activity

The antioxidant activity of normal, silken and intermediate dried tofu coagulated by calcium chloride was found to be 34.52±1.17%, 55.79±1.50% and 30.07±2.10% respectively whereas coagulated by lemon juice was found to be 32.89±1.25%, 42.08±1.24% and 27.85±0.49% respectively as DPPH inhibition.

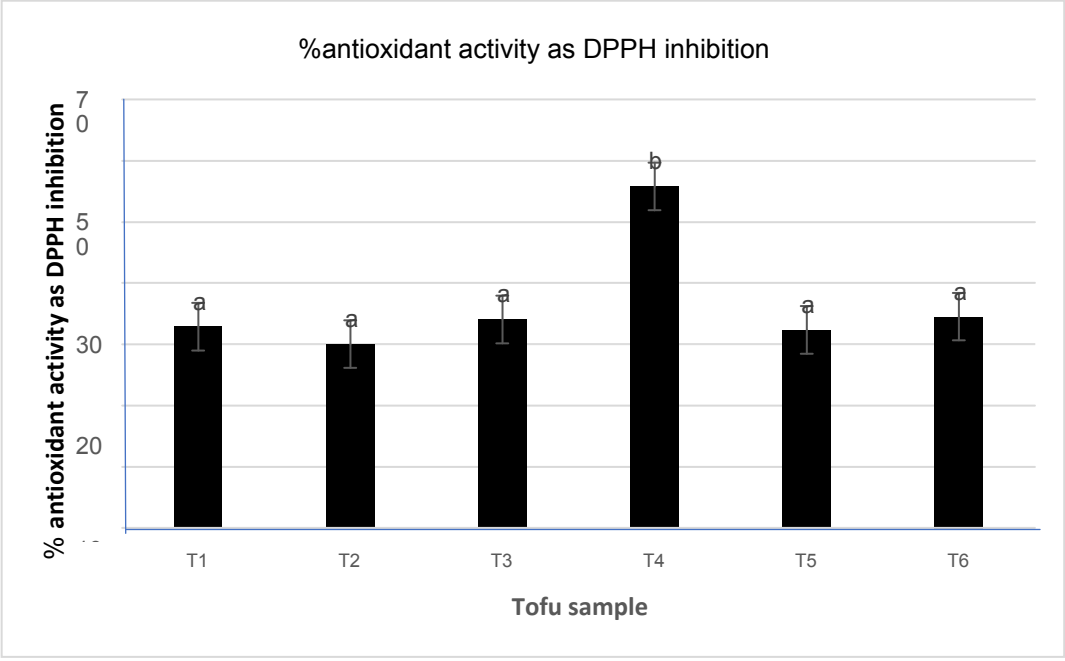

Fig 3. Antioxidant activity of tofu samples

The antioxidant activity of silken tofu coagulated by calcium chloride was found to be the highest indicating high radical scavenging activity. From Statistical analysis (ANOVA), all samples show a significance difference ($p<0.05$) on the antioxidant activity. (**Figure 3**)

Amic *et al.* (2003) has shown that there is a direct relationship between the total phenol content and antioxidant activity, the high phenol content of soybean coagulated tofu may be attributable to the probability that some of the phenols present in soybean may have been transferred to tofu.

Unlike phenols and flavonoids, enzyme- assisted processing has also been reported to have a positive effect on antioxidant activity (Koley *et al*., 2011).

Yield of tofu

The yield of tofu was found to be the highest in intermediate dried tofu coagulated by lemon juice

whereas yield of tofu using calcium chloride as a coagulant was found to be lowest in normal tofu. There is a significance difference ($p<0.05$) between the yield percentage of normal tofu and intermediate dried tofu and also with normal and silken tofu as shown in **Table 2.**

Sanjay *et al.,* (2008) reported use of natural coagulant increased the yield. Similarly, Shokunbi *et al.,* 2011 reported normal tofu prepared by using synthetic coagulant has lower yield.

The yield of tofu depends on different factors. Hou *et al.,* (1997) reported that the coagulants, stirring time and speed had a significant influence on yield and textural properties of tofu. The yield of tofu was also found to be influenced by heating time (Noh *et al.,* 2005).

Depending on yield, there was a significant difference ($p<0.05$) between the coagulant used i.e. calcium chloride and lemon juice depending on the Statistical Analysis (ANOVA). Because lemon juice is a natural coagulant, it has been selected as a best tofu prepared from it. Compared to other types of tofu, the average value of intermediate dried tofu coagulated from lemon juice was highest (36.21±0.65). As a result of this analysis, intermediated dried tofu coagulated with lemon juice was taken as the best tofu.

Table 2. Yield of Normal, Silken and Intermediate dried tofu prepared from 200g soybean

Normal Tofu	% Yield of tofu (on db)
T1	28.29±0.22
T2	22.94±0.65
T3	32.85±0.82
T4	24.01±0.23
T5	36.21±0.65
T6	29.06±0.55

*Data are expressed as mean± standard deviation

**T1: Lemon juice coagulated Normal tofu; T2: Calcium chloride coagulated Normal tofu

**T3: Lemon juice coagulated Silken tofu; T4: Calcium Chloride coagulated Silken tofu

** T5: Lemon juice coagulated intermediate dry tofu; T6: Calcium chloride coagulated intermediate dry tofu

Proximate Composition

Moisture content

Normal tofu: deMan *et al.* (1986) reported moisture content of normal tofu ranges from 76-81%. Gartaula *et al.* (2013) reported moisture content of normal tofu using lemon juice was 71.88%. Obatolu (2008) reported moisture content 73.3% and Shih *et al.* (2002) reported 82.84% moisture content using calcium chloride. The obtained values of moisture content of $CaCl_2$ coagulated tofu is comparatively less than the values reported by Obatolu (2008) and deMan *et al.,* (1986). But the value for lemon juice coagulated was in accordance with Gartaula *et al.* (2013). The variation of moisture may be due to the difference in gel network in the tofu as influenced by different anions and its ionic strength towards the water holding capacity of soy protein gel (Shokunbi *et al.,* 2011). Furthermore, the pressing can also play a vital role in the moisture content of tofu.

Silken tofu: Wang *et al* (1983) reported a range of 87-90% moisture content in Silken tofu. The moisture content obtained by using calcium chloride was found to be 86.88% whereas by using lemon juice was found to be 89.93%**.** Though moisture content of both tofu falls within the reported range, this investigation showed that using lemon juice produced tofu with higher moisture content than with $CaCl_2$.

Intermediate dried tofu: Cai and Chang (1997) reported moisture content below 76% in dried tofu. The obtained value using calcium chloride was found to be 55.21% whereas by using lemon juice moisture content was found to be 50.26%. This indicates tofu coagulated by calcium chloride retain higher moisture content than tofu coagulated by lemon juice, when dried at 100°C/ 2 hours. The lower value achieved in this experiment can be theoutcome of drying environment (55°) used.

Protein content

Normal tofu: Obiegbuna J.E *et al.,* (2014) reported 26.25% protein content by using lemon juice and 19.25% protein content using calcium chloride in normal tofu. Protein content of normal tofu coagulated by calcium chloride was found to be 48.92 % whereas lemon juice coagulated tofu was found to be 55.59%. Though investigated values for protein content are higher, the values do correlate with Obiegbuna J.E *et al.,* (2014). This indicated that lemon juice coagulated tofu contains higher protein content than calcium chloride coagulated tofu.

Silken tofu: Protein content of silken tofu coagulated by calcium chloride was found to be 41.00% whereas in lemon juice coagulated tofu protein content was found to be 53.44%. From investigated data lemon juice coagulated tofu contains high protein content than coagulated by calcium chloride. This may be an indication that lemon juice has greater ability to precipitate protein than calcium chloride.

Intermediate dried tofu: Protein content of dried tofu coagulated by calcium chloride was found to be 53.72% whereas lemon juice coagulated dried tofu was found to be 60.08%. Cai *et al*. (1996) reports that dry tofu (freeze dried) contains protein in the range of 55- 58%.

As tofu being a source of protein, the highest protein content was obtained in intermediate dried tofu coagulated by lemon juice i.e. 60.01±0.02% compared to other types of tofu.

Using lemon juice as a coagulant led to significantly higher protein content in the tofu compared to using $CaCl_2$.

Fat content

Normal tofu: The fat content in lemon juice coagulated tofu were found to be 13.85% whereas by using calcium chloride were found to be 10.67±0.018 in normal tofu. Obiebuna *et al.,* (2014) reported 18.40% fat content in lemon juice and 16.20% fat content in calcium chloride in normal tofu (**Table 3**). Though the fat contents were lower than the reported values, investigated data on fat content of normal tofu correlates with Obiebuna *et al.,* (2014) i.e. fat content is higher in lemon juice coagulated tofu than by calcium

chloride. The discrepancy in the fat content of the raw material can also lead to the difference in the values obtained.

Silken tofu: The fat content in lemon juice coagulated silken tofu was found to be 11.02% whereas calcium chloride coagulated silken tofu was found to be 7.07% (**Table 3**). Investigated data indicates silken tofu coagulated by lemon juice contains higher fat content than calcium chloride coagulated silken tofu.

Intermediate dried tofu: The fat content in lemon juice coagulated dry tofu was found to be 21.65% whereas calcium chloride coagulated dry tofu was found to be 20%.

Overall, calcium chloride coagulated tofu has got lower fat content than coagulated by lemon juice. Gavidel and Prakash (2007) reports that the loss in total solids may impart the reduction in fat content and varietal difference of soybean.

Crude fiber

Normal tofu: Crude fiber content in lemon juice coagulated normal tofu was found to be lower than that of calcium chloride coagulated normal tofu. This could be due to the type of coagulant and amount of coagulant used.

Silken tofu: Crude fiber content in lemon juice coagulated silken tofu was found to be lower than that of calcium chloride coagulated silken tofu.

Intermediate dried tofu: Crude fiber content in lemon juice coagulated dry tofu was found to be lower than that of calcium chloride coagulated tofu. This might be due to difference in types of coagulant.

1. Ash content

Normal tofu: Total ash content in lemon juice coagulated tofu was found to be lower than that of calcium chloride coagulated tofu. This might be due to varietal difference of soybean, amount of coagulant used and moisture content.

Silken tofu: Total ash content in lemon juice coagulated silken tofu was found to be lower than that of calcium chloride coagulated tofu. This might be due to types of coagulant and amount of whey content in silken tofu.

Intermediate dried tofu: Total ash content in lemon juice coagulated tofu was found to be lower than that of calcium chloride coagulated tofu. This could be because of types of coagulant and amount of coagulant used.

2. Carbohydrate

Carbohydrate was calculated by difference method for different types of tofu. Carbohydrate content is higher in calcium chloride coagulated tofu than that of lemon juice coagulated tofu in all three types of tofu. This could be because of different processing method. Similarly, carbohydrate is higher in silken tofu coagulated by calcium chloride. This could be because of amount of whey content in silken tofu.

Table 3. Proximate composition of Normal, Silken and Intermediate dried tofu

Proximate composition for normal, silken and intermediate dried tofu processed with two different coagulants (g/100g Dry basis)

	Normal Tofu		Silken Tofu		Intermediate dried Tofu	
	LJ	CaCl2	LJ	CaCl2	LJ	CaCl2
Code	T1	T2	T3	T4	T5	T6
Moisture (%)	71.12±0.02	62.74±0.03	89.93±0.01	86.88±0.02	50.26±0.02	55.21±0.01
Crude Protein	55.59±0.04	48.92±0.02	53.44±0.04	41.00±0.02	60.08±0.02	53.21±0.01

Crude fat	13.85±0.05	10.67±0.01	11.02±0.04	7.07±0.04	21.65±0.03	20±0.01
Crude fiber	5.03±0.07	5.34±0.06	3.29±0.05	3.23±0.05	3.99±0.05	4.55±0.05
Ash	2.92±0.06	2.06±0.06	1.06±0.04	1.49±0.02	1.35±0.04	2.49±0.06
Carbohydrate	22.6±0.02	33.01±0.07	31.19±0.02	47.21±0.02	12.93±0.02	19.24±0.02

*Values are means± standard deviation of triplicate determination.

**Except moisture content other parameters are in dry basis

***LJ: Lime juice $CaCl_2$: Calcium Chloride

****T1, T2, T3, T4, T5, T6: Codes assigned to various types of Tofu

Sensory evaluation

Sensory evaluation of prepared tofu sample T1, T2, T3, T4, T5 and T6 tofu was carried out for color, texture, taste, appearance and overall acceptability by 10 semi trained panelist (students and staffs of NCFST) using 9 hedonic rating (1= dislike extremely, 9 = like extremely) described by Ranganna,2009.

Sensory Analysis

Color

Normal tofu coagulated by calcium chloride had highest color score (7.20±0.63) and silken tofu coagulated by lemon juice had lowest color score (3.90±0.56).

Texture

Normal tofu coagulated by calcium chloride had highest score for texture (6.40±0.5) and silken tofu coagulated by lemon juice had lowest score for texture (5.20±0.92).

Taste

Silken tofu coagulated by calcium chloride had highest score for taste (7.30±0.48) and likewise, silken tofu had lowest (3.70±0.48).

Appearance

Normal tofu coagulated by calcium chloride had highest score for taste (7.40±0.69) and likewise, silken tofu coagulated by lemon juice had lowest (4.50±0.70).

Overall acceptance

Normal tofu coagulated by calcium chloride had highest score for overall acceptance (7.60±0.96) and likewise, silken tofu coagulated by lemon juice had lowest (4.62±0.96).

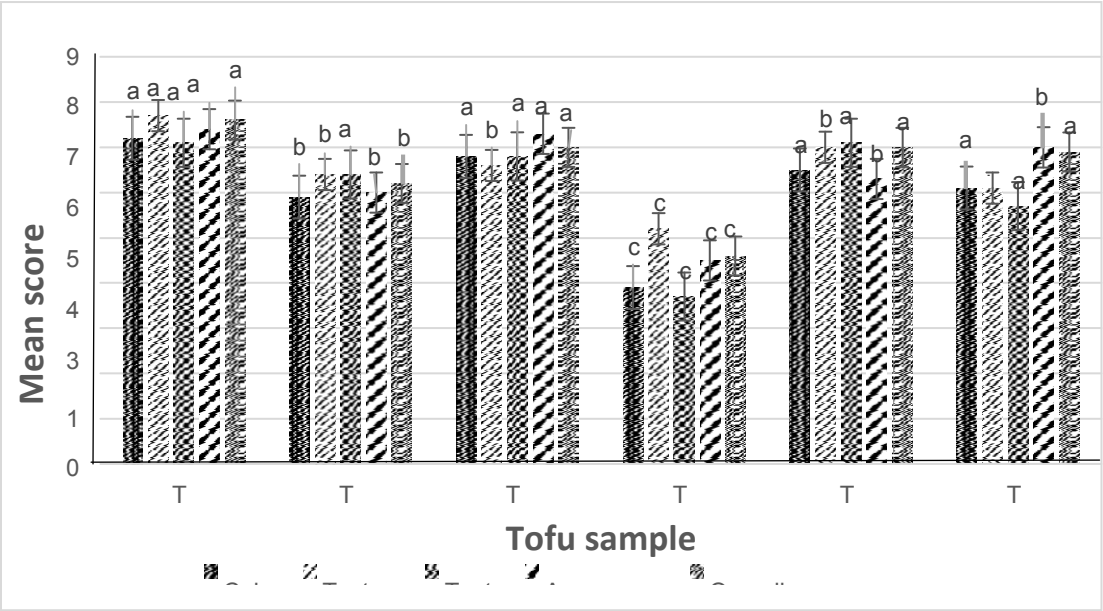

Fig 3: Graph of sensory evaluation on different sensory parameters

Conclusion

Lemon juice as a natural coagulant results significant difference in proximate composition of three different types of tofu comparing it with synthetic coagulant. There was increase in protein content and fat content in all three types of tofu that is normal, silken, and intermediate dried tofu by using lemon juice as a natural coagulant. There was significant increase in yield and sensory properties of tofu's coagulated by natural coagulant in comparison with synthetic coagulant.

Natural coagulant helped in increasing oxalate content alternatively decreasing in phytates content as an antinutritional factor. Additionally, there was decrease in total phenolic content and antioxidant activity in tofu's coagulated with natural coagulant

Hence, it has been confirmed the feasibility of replacing

commercial salts by lemon juice for the coagulation of soybean milk in the manufacture of tofu from black soybean.

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