



**STUDY TOWARDS THE SYNTHESIS AND MICROBIOLOGICAL
ACTIVITIES OF TOFU WITH TURMERIC POWDER**

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ABSTRACT

According to an Indian nutrition survey, most nutrients, particularly protein, are insufficient in the rural population's diets. As the world's fifth-largest breeder of Soybean, a protein-rich crop, India has the potential to solve protein-energy malnutrition by broadening Soybean uses and producing high-values and wellness food items. Tofu's, a shelf-stable Soybean products high in proteins, B vitamins, and isoflavoness, might be a good meat alternative in Indian cooking. Because of its high protein content, tofu has a limited shelf life. To try to lengthen the shelf life, extract of turmeric (*curcuma longa*), which are normally available in rural areas, were utilised. Traditionally, $MgCl_2 \cdot CaSO_4$ was used to coagulate tofu. Aqueous extract containing *curcuma longa* (turmeric) were added to tofu throughout the manufacture and storage process to enhance its shelf life. There was no micro flora in the water used in this study, the plant extract had quite a mesospheric counts of 2.527104 CFU/g, and no yeasts or moulds were identified. Turmeric extract was firmer than control tofu and held 76.4 percent moisture. Despite the fact that there was no discernible difference in mesospheric count among respect to control samples during storage, medicated tofu remained organoleptically acceptable until the case's conclusion, with lower lipid oxidation and 50% (4.7 units) less kinase behaviour within a week of 7 days than control (9.6 units). Overall shelf life of turmeric extract was effectively increased from 3 to 4 days in usual storing without refrigeration to 8 to 10 days using extracts from naturally accessible, easily cultivable turmeric.

Keywords: Microbiological Activities, Tofu, Turmeric Powder, food

1. INTRODUCTION:

Children in rural areas of impoverished countries like India are particularly vulnerable since the food available to them is neither adequate nor optimal for sickness tolerance or physical development [1]. The National Nutrition Monitoring Bureau and the National Institute of Nutrition conducted food and nutrition surveys in 12 Indian states, finding that the rural population's diets are low in essential minerals, especially calories, vitamins, in addition to others nutrients, especially proteins (Vijayaraghavans and Rao 1998) [2]. Cereals, particularly rice, provide over half of the proteins in the average Indian diet today. Because this cereals is low in protein, it's a good idea to eat it with milk. It must be supplemented with foods that are high in protein (Franke 1961) [3]. Soybean seeds contain a protein concentration off 35s to 40% by dryer weights, making them a reasonably affordable sources of protein (Derbyshire and otherse 1976) [4-7]. Because to such isoflavvones (genisteine, daidzeine, and glyciteine) included in Soybean (Fukutakee and others 1996s), Soybean goods have anticarcinogenics, bloods cholesterols, and sugars off reducing properties, which is regarded crucial to cure osteoporosis & among women's due to hormonal changes (Fukutake and others 1996) [8].

Because of their great nutritional values and lows cost, soybean products are a viable cure for malnutrition issues in impoverished people who eat grain-based diets. Tofu has been suggested as a viable answer to the world's food problems since Soybeans produce simultaneously high-quality protein as well as a high output of protein per acre [9-12]. For many years, tofu has been a popular protein source throughout Asia. Tofu is a well-known Soybean food that isn't fermented (curd). Tofu was invented two thousand years ago by the Chinese ruler of the His-Han Dynasty, according to legend. Tofu is becoming more popular in Asian countries as well as the United States (Hsu 1978) [13].

For decades, the same basic processes have been used to make tofu. Soybeans are cleaned and crushed after being soaked until thoroughly dehydrated. The curds are separated and pressed into rather hard square blocks after coagulants are introduced to the soymilk (Snyder and Kwon 1987) [14-17]. Because of its higher protein content and high moistures level, tofu is an exceptionally attractive substrate for microorganisms. Tofu's polyunsaturated to fatty acids (PUFAs) are particularly vulnerable to the oxidations of free radicals and other highly reactive species [18].

As previously mentioned, tofu may be conserved in a variety of ways to increase its

shelf life. Tofu made from ozone-treated Soybeans (Park and others 1994) [22], tofu made from electrolyzed water (EW) (Zhao and others 2002), tofu wrapped in PE films coated with of nosing-incorporated MC/HPMCs (methylcellulose's—MC and hydroxypropyl I methylcellulose—HPMCs) solution (Cha and others 2003), and tofu correlated with utmost pressure (Cha and others 2003). (Prestamo and others 2000), and tofu treated with microwave (Wu and (No and Meyers 2004). The use of acetic acids as a coagulant was also mentioned as a simple preservation strategy Natural coagulants, such as fruit juices, frequently used chemical preservatives in immersion solutions, and simple procedures, such as burning the tofu, are further options (Lee and others ii 1990; Kimu and Liee 1992). (Pontecorvoo and Bournes 1978; Miskovskye and Stonee 1987; Lee and others 1990). Unfortunately, as a result, [20-22] the quality of the tofu produced will diminish. Despite its popularity now, refrigeration is not available in India's villages. If undesired bacteria are present, freezing alone will not eradicate them from tofu, and psychrophilic microorganisms pose a risk of deterioration. Rural dwellers, on the other hand, cannot use any of these strategies owing to high expenses, scarcity, and negative consequences [23]. As a result, natural antibacterial agents should be used in tofu preservation.

Curcuma longa, also known as sacred turmeric, has been utilised in Ayurveda medicine in India for two hundreds of years and is claimed to have antibacterial, anticancer, antioxidant, and hypolipideemic properties [24].

According to the FDA, turmeric is GRAS (generally recognised as safe). Curcumin, demethoxycurcumin, and bisdemethoxycurcumin, which are active components in turmeric, have been found to be cancer-fighting [25]. Turmeric is abundantly accessible and easy to cultivate in rural regions. Tofu may be enriched with physiologically active compounds from a variety of sources, in contrast to is flavones, for human health benefits. The current study looks over the use of curcuma longa extracts as a preservative in tofu making and the use of copper pots for tofu preservation because of turmeric's long history of usage and documented medicinal advantages [26].

2. MATERIALS AND METHODS

Local markets provided Soybeans (*Glycine max.*) and curcuma longa (turmeric). All of the trials were carried out twice. Qualigens chemicals in Mumbai, India, provided the magnesium chloride or calcium sulphate utilised in the coagulation process. The substances employed in this experiment were all of analytical quality.

Preparation of aqueous extract of turmeric:

A technique for making aqueous turmeric extract was developed by Ganasoundari and colleagues (1998). *Curcuma longa* was bought at a marketplace, washed, dried inside the shade, and ground in a mortar and pestle. This powder was used for extraction. Twenty grams of powder were extracted utilizing 150 mL distilled water for one hour at 75°C, cooled, and filtered. The extraction was done four times using a Rotavapor, then pooled and concentrated to 150 mL. (Switzerland, BUCHI). This extract was stored and used in research.

Preparation of tofu:

Soybeans (300 g) were first soaked overnight at room temperature (28°C) in water containing 3% (w/v) turmeric extract, then drained, washed, and crushed for 1 minute at high speed in a mixer grinder with 2000 mL water (Soybean to water ratio=1:8). To remove the beany taste and anti-nutritional components, the resulting slurry was boiled indirectly for 10 minutes. The slurry was

then strained twice through cheese clothes to yield soymilk and pressed cake (Okaras).

A Digital Refractometer 4(RX-500, ATAGO, Co., Ltd., Japan) was used to assess the solid content of soymilk, and the Brix (solid content) was adjusted to 6 - 8 degrees Brix via warming fresh milk over 15 minutes before utilizing the soymilk to create tofu. 1400 mL soymilk was heated to a boil. In 70 mL of water, the blended coagulant (0.2 percent CaSO_4 and 0.23 percent MgCl_2) was prepared. Turmeric extract (5%) was slowly stirred into the boiling milk for a few minutes before being left to coagulate at room temperature for 16 minutes.

That curd was transferred carefully to a ventilated closed vessel (12.0 x 12.0 x 4.0 cm) coated with the double layer of cheesecloth and pounded for 15 minutes with 2 kilogram bricks. After separating the whey, the tofu block was sliced into 2.0 x 2.0 x 2.0 cm pieces.

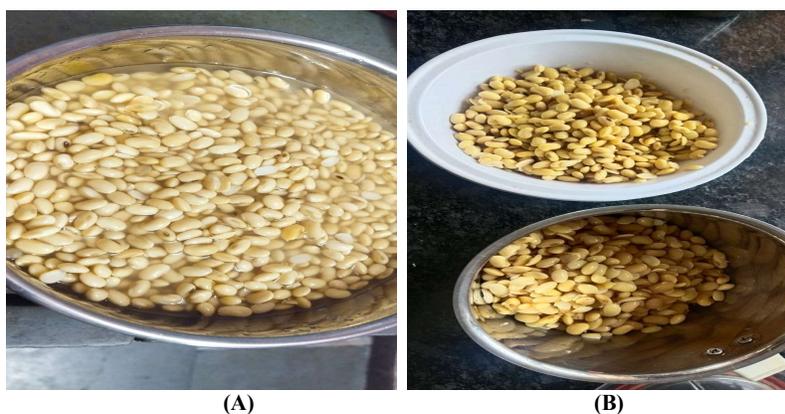




Figure 1: Method of preparation of tofu with turmeric powder

Storage studies:

Prior to usage, the copper containers in this investigation were cleansed with sterile water and dried. The following conditions were used to conduct a tofu storage study: Tofu chunks were submerged in water containing 1% turmeric extract in copper pots. Every 24 hours, the water was changed with new water that contains 1% turmeric extract. Microbiological examination, texture, and peroxidation rate were all performed on tofu samples. Till the last day (7th day) samples, the quantity of

proteolysis was determined. All trials were duplicated and compared to tofu that served as a control.

Chemical analysis

pH:

The pH of tofu samples was measured using a digital pH meter (APX-175E, CD Instrumentation PVT Ltd., Bangalore, India) with a glass electrode. Using a mortar and pestle, ten grams fresh tofu's and 35 mL of deionized water was homogenised, and filtering through a single layers of cheese

clothes to determine pH. (Noe and Meyerse 2004).

Moisture:

The moistures contents of 5 g of tofu was measured by drying it to a constant weight inside a hot air oven (Thermite) at 70°C (AOAC1990).

MICROBIOLOGICAL ANALYSIS:

I Tofu samplers were tested for aerobic mesophilic plates counts, yeast and moulds, coliforms, to and coagulasey-positive *staphylococci* using methods from the Compendium of Approaches for the Microbiologically Examination of Foods, with minor changes (Downs and Ito 2001). Tofu (5 g) was mixed with 45 mL peptone water 0.1 percent (101 dilution) and serially diluted using peptone water up to 1010 dilutions (0.1 percent). Appropriate dilutions have been chosen from these for plating.

Total aerobic plate counts:

Every dilution were dip mounted in parallel on spread plate agar (PCA;s HI media) and incubated for 48 hours at 38 degrees Celsius (Bacterial Incubatory with Air To circulate, Sri s Rudran Instruments, Chennai, India).

Yeasts and moulds:

Each of dilution was deposited in duplicates using HI media rose Bengal chloramphenicol agar was cultivated for 5 days at 30°C.

3. RESULTS AND DISCUSSION:**pH:**

Tofu's pH changes during storage as a result of breakdown or deterioration by microbes. According to Fujii and colleagues (1978), the proliferation of bacteria promotes fast protein breakdown and a pH shift. Because of its highly proteins and moisturise content, tofu is an ideal substrate for microorganisms. When r the pHs of the tofu's samples was measured, as initial pH of normal tofu and turmeric-treated tofu was 6.89 and 6.91, respectively, and a considerable drop in pH was seen in either control (6.21) and turmeric-treated tofu (5.90) after 24 hours. Between 24 and 48 hours, the logarithmic stage of bacterial development might have resulted in successful nutrition use, acid generation, and a significant drop in pH. After 2 days, pH in control and turmeric-treated tofu steadily climbed reached (6.92) or (6.73) just at end of 7 days, respectively. During storage, overall pH of turmeric-treated tofu remained lower than that of control tofu. According to Tuitemwong and Fung (1991), Depending on the sort of invading microorganisms, the pH of tofu climbed or fell. On day 1, 45 percent of these bacteria's tested were Gram-positives when the pH of the tofu was between 4 and 6.

Moisture content:

The moisturise content of the control tofu changed, with fresh tofu containing 70.37 percent water and indicating a 5% increase after 7 days of storage (75.22 percent).

Tofu's gel structure would have been reduced by protein breakdown and lipid globule oxidation, enabling additional water to be absorbed. The moisture content of turmeric-treated tofu (76.49%) fell on the first day (75.21%), then steadily rose until the conclusion of the 7-day period. However, the moisture content scarcely increased by 1% after 7 days of storage. (It's 77.30%).

Microbiological analysis:

We picked turmeric and copper vessels as natural preservatives since they are popular in rural regions. The microbiological load of turmeric extract is 2.527×10^4 CFU/g. Because it is a basic method for keeping tofu in rural areas, the extract was still not sterilized by autoclaving prior to do storage experiments. **Table 1** shows the fluctuation in microbiological counts of the tofu's stored in coppers pots. Control samples revealed as gradually increased in aerobic i count for the first 6 days, so a 1 log decrease at the end of the seventh day. Turmeric-treated samples exhibited a similar trend until the conclusion of the 7-day period, which might be attributed to the turmeric extract's initial load.

Pontecorvo and Bourne (1978) found that freshly cooked tofu contained 3.0×10^4 CFU/g, which was reduced by 1.12×10^3 CFU/g after 10 days at 24°C in 4% NaCl & 10% lemon juice. In the experiment, no coliforms were detected in any of the

samples. The copper pots employed in this investigation are thought to have played a key role in removing *E. coli* from the tofu. Copper leaking into in the water from brass killed *E. coli*, according to researchers from Northumbria University in the United Kingdom and Punjab University in India (News Scientist 2006), they results of Noycer and others (2006) [21] clearly demonstrated these antimicrobial properties of cast copper of alloys with regards to *E. coli* O157, and Recent research has discovered both metallic copper and copper to alloys exhibit antibacterial action against the pathogenic *E. coli* O157:H7 bacteria (as cited in Faundez and others 2004). Szabo and colleagues (1989) observed similar results, with undetectable *E. coli* levels in 80.0 percent of completed tofu samples in their investigation. According to Rehberger and colleagues (1984) [25], verified coliform levels of 1×10^3 CFU/g or higher were found in 67 percent of commercial tofu samples tested. On Baird Parker agar, no distinctive black colonies of *Staphylococcus aureus* grew, as verified by Gram staining. The antibacterial effect of curcuma against *S. aureus* is most likely due to the presence of phenols, notably thyme. For *Klebisella*, *E. coli*, *Proteus*, and *Staphylococcus aureus*, aqueous extract of curcuma longa demonstrated broader inhibitory zones (Geeta and others 2001) [11]. In the investigation by Rehberger and coworkers

[25], Coagulase level is nil. In any of commercial tofu samples tested, Staphylococci were detected (1984). According to Szabo and colleagues (1989), Staphylococci were found in 5.6 percent of conventional tofu samples with 25 to 250 CFU/g and 1.4 percent with 250 to 2500 CFU/g. The durability of the moulds appears to be fluctuating. After 2 days, yeast and mould counts ranged from 1.0×10^2 CFU/g to 2.33×10^5 CFU/g, with a 2-log drop after 4 days and an increase to 2.33×10^5 CFU/g at the end of 7 days. Variations in yeast and mould counts might be due to changes in the number of spores there in air, sample handling during storage, and the impact of turmeric extract on yeast and mould vitality. Because moulds are efficient users of plant

nutrients, turmeric-treated tofu had a higher number of colony forming units.

Rehberger and colleagues (1984) [25] discovered that yeast and mould numbers Vol. 72, Number 8, 2007 —Journal of Food Science M303 M: Food Microbiology & Safety Tofu preservation... was extremely poor with all commercialized tofu pieces (ranging from 10 to 1.50×10^3 CFU/g). Employing dairy principles to soy products may not always be appropriate due to variations in the types of bacteria expected to arise in these two sources. With a bacterial load that would be considered excessive in an animal diet, vegetable meals may be appropriate. The lack of sufficient microbiological standards makes assessing the microbial quality of tofu challenging.

Table 1: report of analysis of tofu prepared from turmeric fat, protein, carbohydrate, cholesterol, dietary fiber, E coli, total yeast and mould count

Sr. No.	Quality Characteristics	Units	Test Method	Result	FSSAI Limits
1	Fat	g/100g	CI.10 of IS 6287:1985 RA2015(Page-13)/Appendix B of IS:4079:1965 RA2011(Page 7)	3.7	-
2	Protein	g/100g	CI:11 of IS:6287:1985 RA2015(page-17)	15.8	-
3	Carbohydrate	g/100g	FAO Chapter-2,2003	13.41	-
4	Total Yeast and Mould Count	cfu per gm	IS 5403:1999 RA 2018	41000	-
5	E.Coli (Detection)	Per 25 gm	IS:5887(P-1):1976 RA 2018	Present	Absent
6	Dietary Fibre	g/100g	FSSAI Manual 2016	7.84	-
7	Cholestrol	Mg/100gm	FSSAI Manual of methods of Analysis of Foods of Oils&Fats,2016 (GC Method)	0.0	-

If criteria are established, it must consider the microbial flora found in genuine tofu samples, as well as the risk that these organisms might endanger customers' health. It is not adequate to "steal" criteria from another product and apply them to tofu

since the types and amounts of organisms that create a danger in tofu and other foods are different. There are no guidelines for tofu preparation because it is a traditional dish. More inquiry is required.

Proteolysis

Proteolysis is a hallmark aspect of bacterial deterioration that causes a foul and disagreeable odour to develop. Protease enzyme activity in tofu samples was measured to assess the rate of proteolysis. Despite the presence of more micro flora in turmeric-treated tofu, only half of a proteolysis activity (4.7 units) has been seen after 7 days of storage as compares to the control tofu (9.6 units). Tofu is stored under coppery pots demonstrated higher protease activity than tofu not conserved in copper pots (9.6 units). This might be due to the inhibitory/antimicrobial activity of turmeric extractive or the protease n enzyme produced might have been inactivated through turmeric treatment.

4. CONCLUSION

The purpose of this research was to increase the shelf life of proteins-rich tofu thus that it might be consumed in developing countries' such as India to address protein-energy malnutrition. Turmeric extract-infused tofu was smoother and had a stronger turmeric flavour. Despite the fact that the microbial burden was equal to that of control tofu, treated samples had lower protease activity, suggesting that many of the microorganisms really aren't proteolysis and that photolytic bacteria were specifically suppressed by turmeric extract. Only nonproteolytic microorganisms made it through the experiment. The use of turmeric extracts resulted into fur with a softy texture that

retained its taste, fragrance, and pleasing overall look, which is also popular among Indians because we employ green herbs in our cooking. It is possible to keep good tofu development and acceptable tofu quality in rural areas without needing refrigerated for more than 7 days by using readily available natural sources including such extracts of the region's curcuma longa.

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