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ASSESSMENT OF MICROBIOLOGICAL QUALITY AND ORGANOLEPTIC CHANGE ON STORAGE DURATION OF STANDARDIZED COOKIES PREPARED BY BLEND OF LOTUS STEM POWDER WITH REFINED WHEAT FLOUR FOR DETERMINATION OF ITS SHELF LIFE

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Keywords:

Microbiological quality, Lotus stem, Cookies, Sensory quality, Enumeration, Polyethylene, Colony forming unit (CFU).

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ABSTRACT: Incorporating the under-consumed super food ingredients rich in nutrients or functional compounds is a growing concern in healthcare product formulation. Health-ensuring cookies blend with *Nelumbo nucifera* (Lotus) stem powder were standardized in the laboratory and found its acceptable form with comparatively higher acceptability and good flavour profile. Safety assurance is also a part of product development to assure and instruct the consumer regarding its life span or safe consumption time on storage duration. Therefore, the study further aimed at to- Aims and Objective: 1. To assess the quality of lotus stem cookies in terms of microbiological and organoleptic aspects. 2. To assess the impact of the growth of microorganisms in cookies prepared through blend of lotus stem powder. **Methodology:** Keeping quality of this formulated product has been assessed by enumerating the total bacteria, yeast and mould count in between a fixed time duration (0 – 90 days) and assessing its sensory qualities. AOAC (1994) suggested techniques (microbiological count assessment) and 9 point hedonic scale to observe the flavour profile were used for this purpose. Obtained data had been statistically analyzed using ANOVA, t-test statistics *etc*. **Results and Discussion:** The microbial growth rate had increased at high rate after 3 months as total plate count (TPC) was enumerated. The initial count of bacteria of freshly prepared cookies was 0 cfu/g and during 90th days of storage, the bacterial activity was increased, and their count was obtained as 4.6×10^5 cfu/g (mean value of TPC). It comes under the range of borderline count of safe limit and indicates that the microbial status of the product found to be safe according to its standard value. At the same time, the mean value of yeast & mould count of these fortified cookies was 0 cfu/g on the day of preparation, which increased to o 4.8×10^4 cfu/g on the 90th day of storage. Because of the polyethylene (0.11 mm thickness) packaging and sealed by hand sealing machine and low moisture content of the product, their yeast mould count had risen under safe limits on <90 days during storage. As p-value (<0.05), the cookies prepared by incorporating lotus stem had better microbiological quality than control cookies. The sensory quality analysis of the product revealed that the mean scores of colour, texture, taste, flavour and overall acceptability attributes showed minutely decline scores during storage days. Still, these scores were stable in very good (8) to good range. It may be due to increased microbial activities on 90th day of storage. **Conclusion:** The association of days of storage duration with increments of total viable numbers was showed statistically significant at 5% level. Therefore it can be concluded that the quality of product determined as <90days had good acceptability and safety from the consumption point of view.

INTRODUCTION: The value addition of cookies with simple ingredients is a simple and feasible method of enhancing these foods' nutritional value and adding variety to diets.

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Formulating new flavored and health concerning food products by incorporating super food having functional qualities is a technique that provides a variety of ready-to-eat healthy foods [7].

At the same time, these products keeping period and storage time are a matter of safety from health hazards. Therefore, keeping time is carefully determined to ensure the count of microorganisms in products, and whether they are stable under the safe limits is decided by quality control authorities during and at the end of storage day. As WHO had

recommended the safe limit of total bacteria (2.0×10^5 cfu/g), specific bacteria yeast, and mould count (1.0×10^4 cfu/g) for baked products [12]. When the product has a safe count of microorganisms in terms of bacteria, yeasts and mould, it refers to having good microbiological quality. As the level of moisture that increases after a long or short duration (depends on nature of the product) the growth of yeast and mould and then bacteria accelerates, making it unsafe and producing compositional degradation, leading to organoleptic changes and making it unacceptable. Before coming to this situation, this period is indicated as its shelf life, keeping period, or storage time. Their determination is necessary after product formulation using chemical, microbiological and organoleptic tests.

Indian systems of therapies and medicine (Ayurveda, Unani, Siddha, Yoga and Naturopathy) are primarily centers where medicinal foods or herbal medicines are gaining more importance in treating different diseases because the modern synthetic medicines have many side effects. Innovations are going on in the area of nutraceutical and healthy foods. People are fed up with pharmacy-based treatment and nutritional supplementation, so they moved to our traditional foods (having functional quality with medicinal effects) for prevention. But they have a hectic schedule and no time to balance meal preparation in a day; therefore, they depend on food and nutrient supplements. Hence, one of the food industry's efforts is to supply nutritionally balanced foods with some health claims, safety assurance and maximum consumer satisfaction.

On this line of innovation, this study aimed to conceive a new food product using ingredients i.e., lotus stem powder to blend it with refined wheat flour to make cookies because cookies are a more popular snack food. It refers to a baked product generally containing three major ingredients viz; flour, sugar and fat, mixed with other minor ingredients to form a dough. Cookies are good carriers of nutrients like carbohydrates and fat, which can be enriched with lotus stem (*Nelumbo nucifera*) powder replacing refined wheat flour up to an acceptable level. The shelf life of the acceptable cookies prepared by blend flour depends on their total viable numbers on post preparation, increases during storage duration and responsible to produce deterioration. The total viable count of any prepared food product increase during storage is due to post-processing contamination [1]. At the same time the nature of incorporate ingredients also can be a factor for the same, so that the microbiological quality assessment of most acceptable cookies can be measured and that determine the storage duration or keeping time.

About the Lotus Stem: A perennial aquatic basal eudicot with two species, the lotus (*Nelumbo nucifera*), is a member of the small family Nelumbonaceace. It is a key horticultural plant that has been extensively used, particularly in Southeast Asia, for purposes ranging from ornamental, nutritional to medicinal. The scientific community has recently been giving a lot of attention to the lotus. It has been the subject of an increasing number of studies, which have revealed some of this species' mysteries [14].

Nelumbo nucifera belongs to the family Nelumbonaceae, commonly known as Indian lotus, Bean of India, Chinese water lily, and Sacred Lotus, and there are several related species like *Nelumbium nelumbo*, *N. speciosum* and *Nymphaea nelumbo*. On the dietary aspect, almost all parts of the plant are used in preparing diverse cuisines. *N. nucifera* is a large aquatic rhizomatous herb consisting of slender, elongated creeping stem with nodal roots, aerial and floating orbicular leaves. Its petioles are considerably long and rough with distinct prickles. Flowers vary in colour from white to rosy and are pleasantly sweet-scented, solitary, and hermaphrodite.

Tuberous roots are 8 inches long and 2 inches in diameter. The smooth outer skin of the lotus root is green in colour; however, the inner part possesses numerous big air pockets running throughout the length of the tuber assisting for floating in the aquatic system [7]. *N. nucifera* contains some thermal-stable proteins that might be useful in protein bioengineering processes. The proteins are characterized by seed longevity used for cell protection and repair under stress. There are also several indications that compounds of *N. nucifera* are used in drug fabrication in human health research for multiple purposes.

Lotus stem is used for treatment of haematemesis, epitaxis and haematuria, it is also seful in treating diarrhea, cholera, fever and hyperdypsia and have purported diuretic, anti-diabetic and anti-inflammatory properties.

Therefore, in the beam of above scientific proceedings, it was selected to standardize the bakery product (cookies) based on substitution of refined wheat flour by dry lotus stem powder in present research with assessment of their keeping quality.

MATERIAL AND METHODS:

Sampling: The samples (cookies) were taken and prepared (by grinding the product and taking1gm of sample and made their dilutions for each test) in laboratory for microbiological assay, carried out to assess TPC and yeast and mould count. The packet of cookies was opened on the decided days i.e. 0 to 90 days (0^{th}, 15^{th}, 30^{th}, 45^{th}, 60^{th}, 75^{th} and 90^{th} day) and served in front of the panel members for sensory evaluation and then for microbial assessment.

Organoleptic (Sensory) Analysis of Cookies: Organoleptic evaluation of the product (cookies) was done by 12 semi-trained panel members from Era University, Lucknow on above 7 different days. The sensory quality attributes such as colour, texture, taste, flavor (aroma) and overall acceptability were assessed by nine-point hedonic scale with the help of scorecards.

Microbiological Assessment of Cookies: Total plate count (TPC) and yeast & mould counts of cookies samples was conducted at microbiology laboratory of Chhatrapati Shahu Ji Maharaj University, Kanpur on above mentioned days of storage. Methods suggested by AOAC (2000) 3 were adopted for these tests. The plate count agar media (for TPC assay) and potato-dextrose agar media (for yeast and mould count assay) were prepared and poured into sterilized Petri dishes, then they were carefully kept in the laminar air flow until solidification. The diluted samples were placed in that agar plates. The prepared petriplates were covered and named or labelled. These plates were carefully incubated and observed and colonies were counted after 48 hours of incubation for plate count and yeast and mould count respectively. Then their numbers were calculated as a colony-forming unit (cfu) from formula. The number of yeast mould per gram was calculated by multiplying the number of dilutions with the number of colonies counted; same for total bacterial count was applied.

Statistical Analysis of Data: The data were analyzed in Excel and SPSS by using mean values, analysis of variance (ANOVA), t-test and chi square statistics.

RESULTS: After experiments, the obtained data were statistically analyzed and findings revealed the results that have been presented here in the following manner-

Microbiological Changes Revealed in Cookies during to Storage: The common microbiological contamination has been measured by total viable numbers by testing. Total viable count included TPC and total yeast and mould (fungal) count of the samples of freshly prepared cookies and at 15 days intervals up to 90th day of preparation. Due to high temperature (130°C), the total viable numbers were negligible in baked cookies sampled after cooling and before packaging. Their activities have been shown during the days of keeping period. These are as follows:

Total Plate Count: This is presented in **Table 1** that TPC of selected cookies was slightly accelerated on 15^{th} day of storage i.e. 1.1×10^3 cfu/g, then 1.9×10^3 on 30^{th} day, 2.6×10^3 on 45th day, 2.8×103 on 60^{th} day, 3.4×10^3 on 75^{th} day and 4.6×10^5 cfu/g obtained on 90^{th} day of keeping at room temperature in sealed packets. These counts were under the safe limits as decided by WHO. They determined the duration of cookies prepared with lotus stem powder and assured the bacteriological quality of the product as < 90 days. These findings are supported by the results of study of Agu HO, and Ndidimaka AO 2014, as their wheat-based formulated product has 4.0×10^3 TPC and 5.0×10^4 mould count after 20 days of storage [1]. According to them, it's because of contamination of post-processing or handling, but this is included in minimum microbiological load so that their formulated biscuits have long keeping quality. The present attempt was also made by statistically comparing total bacterial load of fortified and

control cookies to investigate the effect of lotus stem powder in refined wheat flour on bacterial activity in cookies during storage.

It was revealed that there was a non-significant difference ($p > 0.05$) between the control or cookies and lotus stem powder substituted cookies on test days during their storage period but the TPC of both cookies showed statistically significant difference (< 0.05) on 90th day's test on storage duration. This showed that the growth of total bacteria had been found to be comparatively lower in fortified cookies than control cookies; it may be due to low moisture content of lotus stem powder.

Note: 2×10^5 cfu/g is the standard count of maximum safe limit determined by WHO (1994) for TPC.

TABLE 1: SHOWING COMPARISON ON MEAN OF TOTAL PLATE COUNT / G OF COOKIES PREPARED BY LOTUS STEM POWDER (CPLSP) AND CONTROL COOKIES (CC) SAMPLES COLLECTED ON PARTICULAR DAYS DURING STORAGE

Storage time (days)	Mean# values of TPC (cfu /g) of CPLSP	Mean*value of TPC (cfu /g) of CC	p value
0 days	No growth (nil)	No growth (nil)	0.0
15 days	1.1×10^3	1.0×10^3	0.28
30 days	1.9×10^3	2.1×10^3	0.16
45 days	2.6×10^3	2.4×10^3	0.23
60 days	2.8×10^3	2.6×10^3	0.20
75 days	3.4×10^3	3.2×10^3	0.18
90 days	4.6×10^5	4.9×10^5	0. 02*

An average value of three sample's enumeration of same population/mass. TPC: Satisfactory (safe) = $<10^4$cfu / g, Borderline contamination = $10^4 – 10^6$cfu / g, unsafe count= $>10^6$ *significant difference

Total Yeast and Mould Count: Table 2 indicated that total fungi count was 2×10^5 cfu/g in fortified cookies on 15th day from zero. It may be because of handling during packaging, their count has been increased and followed by next 15 days i.e: 1.9×10^3 cfu/g on 30th day; 2.5×10^3 on 45th day; 2.8×10^3 on 60th day; 3.3×10^3 on 75th day; 4.8×10^4 cfu / g on 90th day of storage, but this count is linear and under the safe limits except 90th day. This count has reached the borderline of safe limit. Therefore, the storage time to concern yeast mould count has been determined, i.e. < 90 days.

At the same time the total count differences of yeast and mould of samples of fortified and control cookies were found to be statistically non-significant ($p = > 0.05$) on all selected days of storage period except the 90th day's enumeration that showed the difference on yeast mould count of both type cookies was significant ($p = < 0.05$). This result shows that the fortified cookies have significantly less yeast and mould count than control cookie.

Interpretation: The study on substitution of Bengal gram flour by lotus stem powder (LSP) in sev (a product) revealed that moisture percent of product has decreased with substitution percent of LSP in 3 variations [6].

This finding makes confirm that LSP have low moisture percent than other flour that provides low moisture for microbial activities and affected their count increments in product.

TABLE 2: SHOWING MEAN OF TOTAL YEAST AND MOULD COUNT/G OF COOKIES PREPARED BY LOTUS STEM POWDER (CPLSP) AND CONTROL COOKIES (CC) SAMPLES COLLECTED ON PARTICULAR DAYS DURING STORAGE

Storage time (days)	Mean# values of Yeast mould count (cfu /g) of CPLSP	Mean # value of total yeast & mould count (cfu/g)of CC	p value
0days	No growth (nil)	Nil	0.0
15days	1.1×10^3	1.0×10^3	0.43
30days	1.9×10^3	2.0×10^3	0.36
45days	2.5×10^3	2.7×10^3	0.41
60days	2.8×10^3	3.0×10^3	0.23
75days	3.3×10^3	3.4×10^3	0.68
90days	4.8×10^4	5×10^4	0.01*

An average value of three sample's enumeration of same population/mass. Note: Standard value of Yeast and mould count of dry bakery products is < 1.0 × 104cfu/suggested by WHO (1994). *significant difference.

Organoleptic Changes during Storage: The keeping duration can change (decline) the sensory qualities of food products that depend on many factors like handling, packaging, food composition, etc. It was investigated in different days on the storage duration of cookies prepared by lotus stem powder shown in **Table 3.** No remarkable changes were seen (till 90th day of keeping) in all characteristics of product's sensory qualities during these days of storage and their range was maintained at 'good' score range. But the mean scores of all attributes showed a declining pattern and lower the range of quality from their freshly prepared condition to the storage period. As the table presented, the mean scores of overall product acceptability were 8.08 just after the preparation, followed by 7.86, 7.56, 7.43 and 7.13 on 15th, 30th, 45th and 60th day of storage duration, respectively. The results showed similar findings and found the excellent sensory qualities during 90 days storage period of biscuits prepared by bland giloy stem powder [11].

TABLE 3: MEAN OF SENSORY QUALITIES DURING STORAGE OF FORTIFIED COOKIES THROUGH A BLEND OF LOTUS STEM POWDER

Storage time (days)	Mean score of colour	Mean score of texture	Mean score of taste	Mean scoreof flavour	Mean score of overall acceptability
0days	7.83	8.68	8.58	7.83	8.08
15days	7.75	8.59	8.40	7.64	7.86
30days	7.66	8.33	8.33	7.54	7.56
45days	7.34	8.08	8.00	7.36	7.43
60days	7.25	8.00	7.91	7.00	7.13
75days	7.08	7.41	7.66	6.66	7.05
90days	6.91	6.74	7.47	6.43	7.00

Association between Microbiological and Organoleptic Changes: It is well documented that acceleration of spoilage-producing microorganisms in food causes undesirable changes and decline their sensory quality and effects on scores of organoleptic attributes under their stable count in safe limits during storage is a matter of investigation.

Table 4 shows the association of two variables as the overall acceptability of standard cookies formulated by lotus stem powder and its bacterial load during storage on test days and time. As the time of keeping moved, their total bacterial count (cfu/g) increased linearly. The chi-square test revealed that this association is statistically significant at 5%.

TABLE 4: RANGE OF OVERALL ACCEPTABILITY OF COOKIES (INCORPORATED BY LOTUS STEM POWDER) AND FUNGAL (YEAST & MOULD) GROWTH DURING STORAGE

Storage duration (days)	Mean Scores of overall acceptability of cookies of CPLSP	Mean values of TPC (cfu/g) of CPLSP
0days	8.08	No growth (nil)
15days	7.86	1.1×10^3
30days	7.56	1.9×10^3
45days	7.43	2.6×10^3
60days	7.13	2.8×10^3
75days	7.05	3.4×10^3
90 days	7.00	4.6×10^5
		$\chi 2 = 6.352$

Table 5 presented the of two variables as overall acceptability of standard cookies formulated by lotus stem powder and its yeast and mould count on test day and time during storage. As the time keeping time moved, the total yeast and mould count increased in linear. The chi square statistics revealed that this association is statistically significant at 5% level.

TABLE 5: RANGE OF OVERALL ACCEPTABILITY OF COOKIES (IMPROVED BY LOTUS STEM POWDER) AND FUNGAL (YEAST & MOULD) GROWTH DURING STORAGE

Storage duration (days)	Scores of overall acceptability of cookies of CPLSP	Mean values of Yeast and mould count (cfu/g) of CPLSP
0days	8.08	No growth (nil)

15days	7.86	1.1×10^3
30days	7.56	1.9×10^3
45days	7.43	2.5×10^3
60days	7.13	2.8×10^3
75days	7.05	3.3×10^3
90days	7.00	4.8×10^4
		$\chi^2=24.65$

CONCLUSION: The study has determined that the fortified (by lotus stem powder) cookies have their sensory and microbiological qualities in good and satisfactory range, respectively till 90 days of storage in proper polyethylene packaging at room temperature. Therefore, it is suggested to consume this product <90 days. The reveals of present study also proved that the cookies prepared by blend of lotus stem powder have better microbiological quality than control cookies (refined flour based) in terms of their total viable counts.

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CONFLICTS OF INTEREST: Declared None

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