



HEAVY METALS AND SHELF LIFE STUDY: AN EVALUATION OF METALLIC ELEMENTS AND MICROBIAL COUNT ON COOKIES PREPARED WITH MULTI MILLETS

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ABSTRACT

The present work was done to look over the heavy metal analysis of selective grains used to prepare cookies and shelf life evaluation of developed products. The variations of cookies were divided into 5 groups where T0 was standard cookies prepared with whole wheat flour and T1, T2, T3 and T4 were the experimental cookies prepared with wheat flour and different types of millets flour (Pearl millet, Sorghum millet and Finger millet). The data revealed that the heavy metals were analyzed in grains (mg/kg) was found in permissible amount. Where mercury, arsenic, cadmium, copper, lead, tin and zinc were found in lowest level within the range i.e 0.01,0.02, 0.001, 0.85, 0.003, 0.5 and 9.23 respectively. In shelf life analysis of cookies, the total plate count and total microbial count were analyzed and found it appropriate on room temperature (20°c) during 30 days of storage. The total bacterial counts of the developed cookies were found lowest in sample T1 ranged 200cfu/g to 230cfu/g. The total yeast and mould count of developed cookies was found lowest in sample T1 less than 10cfu/g during 30 days of storage. All the developed products were found lowest count (<300cfu/g and <40cfu/g) during the storage period on room temperature.

KEYWORDS: Millets, Heavy Metal, Shelf life evaluation, Pearl millet, Mercury.

1.INTRODUCTION-

Millets are the native of India and very popular grain also known as 'Nutri Cereals'. In the field of agriculture, modern technologies and progressive scientific knowledge have promoted better life style of mankind. In agriculture selection and development of good quality seeds, technology innovation, organic fertilizers processing, and improvement of irrigation facilities and use of pesticides have proven the availability of food has been increased[1,2]. Due to change the climatic condition production of crops are declining by farmers. Like India, in many developing countries, just because of dry land, subtracting rainfall and low fertile soil, lowering the crop production rate day by day. According to World Bank hunger is a big challenge worldwide [3]. Millet based food products also help in improving the health status of human being. It contains major and minor nutrients in a noticeable amount. It also prevents many diseases and keeps our body healthy. [4]

Millets are the robust plants able to growing in those areas which experience subtracting rainfall. These have been grown in Indian sub continent and Africa. These are the oldest crops grows in southern and northern parts

of Africa and Asia in around 1500 to 2000BC [5].The importance of millets are, It increased food safety and security by reduced the climatic stress because it grows in dry or semi-arid region. The second perspective is that it is rich in nutrients and phenolic compounds which fulfil daily nutritional requirement. The third perspective is that they protect from many diseases as they are gluten-free which is useful in celiac disease, rich in antioxidants and low GI level prevent from heart disease and diabetes. The fourth perspective was profitable and economically very cheap in prize, and low investment crop so farmers can earn more than others [6,7].

Development of food products by using grains or small millets is the vital part to motivate nutritional and healthy habits in community. As it has been noticed that mostly people are seen less interested to take grains in their original form, they always need something changed and innovative food items prepared by millets. According to Raywinger (2006), whenever think about the term 'Product Development' by millets and those are- 'cost deduction', 'Nutritious and variety of food products', 'easily digestible', 'easily available' and new innovation according to taste, age, likes and dislikes of people. All these things matter in community nutrition, because the nutritional level of community has to be improved by intervene them with nutritive and low cost foods [8,9,10]

Nazish Huma Khan, et al (2016) conducted a research on heavy metals in soil and assessed that the grains produced in orchard field soil have low concentration of heavy metals and grain produced in non-orchard field soil have moderate amount of heavy metals such as cadmium (0.08mg/kg) found permissible. **Deepmala Satpathy, et al (2014)** assessed the heavy metals contamination in plants and paddy soils. They founded that there are so many toxic heavy metals releases in paddy soil. And this is affecting health of adults, children and old age persons. They developed a product with millets and found the heavy metals ranges are permissible [11,12,13].

Flora V. Romeo, et al (2010) developed some cookies with almonds and introduced different types of packaging and its impact on the shelf life of the cookies. The cookies were stored in different conditions, temperature and packaging as well. They tested total bacterial count and total fungal count on different temperature in every 7 days, and founded some changes in microbial growth [14].

2. MATERIAL AND METHODOLOGY-

The methodology was done in three phases. The first phase was collection of ingredients, heavy metals analysis of selected grains and standardization of recipe. The second phase was development of product and its evaluation (Shelf life). And the third phase is statistical analysis and result and discussion.

Phase-1:-

2.1- Collection of ingredients and standardization of recipe: - In this phase raw ingredients were collected from the local market of Faridabad, Haryana, India, like different types of millets such as Bajra, jowar and ragi and also add sugar, oil/ghee, milk and wheat flour.

2.2-Heavy Metal Analysis of Millets

The heavy metals of grains analyzed by the reference method of AOAC 2015.01. In this test heavy metals like-mercury, arsenic, cadmium, copper, lead, tin and zinc were analyzed.

2.2.1-Analysis of Mercury

The apparatus used for analysis was mercury analyzer. About 5.0gm of sample put into digestion flask, add 25 ml of H₂SO₄, 20ml HNO₃ and 2% sodium molybdate solution with five boiling chips. Connect the apparatus with water circulating condenser. Apply gentle heat for 1 hour. Remove heat and let it stand for

10-15 minutes. Turn off water circulating condenser and boil on high flame till the white fume appears in flask. Cool solution on room temperature and take reading.

2.2.2-Analysis of Arsenic

Apparatus used for analysis was generators and absorption tubes. Add carbonate solution in the sample and stirring slowly, filter and wash with chilled water and dry at room temperature. Dissolve this solution in pyridine and chill water for precipitated completely. Dry the pale yellow color crystal under low pressure and stored it in amber battle for testing the metal. Add 25-50ml HNO₃ and continuously add 40 ml H₂SO₄ in a flask with sample. Heat slightly till the foaming become excessive cool and dilute with 75ml of water and noted the reading [15].

2.2.3-Analysis of Cadmium, Copper, Lead, Tin and Zinc

The apparatus used was atomic absorption spectrophotometer. Standard solution for-

Lead-1mg/ml Lead in 7 ml HNO₃ in 1 liter volumetric flask in dissolved form.

Cadmium-1mg/ml Cadmium in 14 ml HNO₃ in 1 liter volumetric flask in dissolved form.

Copper- 1mg/ml Copper in 7 ml HNO₃ in 1 liter volumetric flask in dissolved form.

Zinc-1mg/ml Zinc in 14 ml HNO₃ in 1 liter volumetric flask in dissolved form.

Tin-1mg/ml Tin in 7 ml HNO₃ in 1 liter volumetric flask in dissolved form.

Weigh accurately 25 gm of sample of different metal separately; add 25ml of sulfuric acid. Mix thoroughly, dry the content in an oven on 110°c till completely dry. Remove from oven and cool. Obtained ash should be white or brownish. Add HNO₃ and 10 ml of water, heat till sample ash is dissolved. Cool and transfer this content in volumetric flask and noted the reading [16].

2.3- Standardization of Recipe-

2.4- Preparation of Product in different variations by the use of different millets- four types of millets i.e. Bajra, Jowar, Ragi and Combination of three millets were used for making cookies. There will be four variations prepared for including all three millets i.e. T1 (Bajra cookies), T2 (Jowar cookies), T3 (Ragi cookies) and T4 (combination of all three millets) respectively.

2.5- Treatment Combination- The different combination used in the experiments is represented as follow in Table-2.1.

Table-2.1:- Standardization Table of cookies.

Samples	Wheat flour (%)	Pearl millet flour (%)	Sorghum millet flour (%)	Finger millet flour (%)	Peanuts
T0	100	0	0	0	0
T1	20	70	0	0	10
T2	20	0	70	0	10
T3	20	0	0	70	10
T4	30	20	20	20	10

Where –

T0- Wheat flour 100 % with sugar, fat and milk without any value added product.

T1- Pearl millet flour 70%, wheat flour 20%, peanuts 10% with sugar, fat and milk.

T2- Sorghum flour 70%, wheat flour 20%, peanuts 10 % with sugar, fat and milk.

T3- Finger millet flour 70%, wheat flour 20%, peanuts 10 % with sugar, fat and milk.

T4- Wheat flour 30%, Pearl millet 20%, Sorghum millet flour 20%, Finger millet flour 20%, peanuts 10% with sugar, fat and milk.

No. of treatment= 5

No. of Replication=4

No. of trial=20

2.6- Preparation of Experimental and Controlled cookies-

To prepared cookies first we mixed fat and sugar together and whisk it for two minutes to make a fine creamy paste. After that we added different types of millets flour, wheat flour, peanuts, milk and other bakery products separately to make five variations (T0, T1, T2, T3 and T4). The cookie dough kneaded manually to make sure its uniformity. The dough was transferred in a clean and greasy tray and rolled it gently. The dough was cut down in square shapes by biscuit cutter. Greased a tray and place all the cookies in pre-heat oven for 40 minutes on 180°c temperature. The baked cookies were packaged after cooling [17].

Whole Wheat Flour and millet flour (100 gm)

Mix all the Ingredients (Oil/ghee, sugar and milk)

Make a dough (Rest for 20 minutes)

Bake for 20 minutes at 180°C temperature

Cooling on room temperature

Packaging and Storage

Figure: 1 - Flow chart for preparation of control cookies (T0)

*Source: - 'Baked Dishes Cookbook by Tarla Dalal, 2006'

2.7- Microbiological analysis of Product

Microbiological evaluation was to be done on standard cookies and developed cookies for food safety to test their shelf life and check microbial risk factors were determined by the method of Indian Standard: 5401, 5402 and 5403. (Indian Standard of Product analysis, 1973-1989)

2.7.1-Determination of Total Plate Count

Total plate count done by IS 5402 method. Used apparatus was oven, autoclave, incubator, petri dish and flask. Place the sample in a petri dish; pour 15ml of plate count agar at 47°c into dish. Mix it carefully tills solidifies the solution and place the dish on horizontal surface in cool and dry area. Place this dish in incubator on 1°c and count the colonies on 7th day, 15th day and 30th day [18].

2.7.2-Determination of Yeast and Mould

Yeast and mould count was done by IS 5403 method by manual of bureau of Indian standard. In this prepared sample pour in a Petri dish. The melted yeast-extract-dextrose-chloramphenicol-agar medium was mixed and maintained at $45^{\circ}\text{C} \pm 1^{\circ}\text{C}$ in water-bath carefully and allowed to solidify. Cool the solution on horizontal surface and inverted the plate in incubator on $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$. fungal colonies were appearing after incubation and counted on different days and clarified in cfu/g of samples [19].

3- RESULT AND DISCUSSION-

The present study entitled as “Heavy Metals and Shelf Life Study: An Evaluation of Metallic Elements and Microbial Count on Cookies Prepared with Multi Millets” is presented statistically analyzed with data interpretation in this chapter.

Table 3.1: Heavy Metal assessment of Wheat and selected Millets

Parameters	Wheat	Pearl	Sorghum	Finger millet	Permissible standard value (mg/kg)
Mercury(mg/kg)	0.05±0.02	0.01±0.00	0.001±0.01	0.02±0.00	1.0
Arsenic(mg/kg)	0.50±0.01	0.03±0.00	0.02±0.00	0.03±0.01	1.1
Cadmium(mg/kg)	0.04±0.01	0.001±0.02	0.002±0.00	0.03±0.01	0.2
Copper(mg/kg)	3.50±0.12	2.00±0.01	0.85±0.02	2.35±0.13	30.0
Lead(mg/kg)	0.08±0.01	0.003±0.00	0.004±0.02	0.005±0.00	2.5
Tin(mg/kg)	1.00±0.02	0.50±0.01	0.23±0.04	0.19±0.01	250
Zinc(mg/kg)	15.54±0.04	14.51±0.03	9.23±0.01	9.54±0.04	50

Standard value references-PFA (fssai)-2021. [20]

The grains were collected from the local market and analyzed with replications. The levels of heavy metals were evaluated in wheat and various millet grains. The level of mercury, arsenic, cadmium, and copper, lead, tin and zinc in the investigated grains are presented in table 4.1. The concentration of mercury in grains ranged from (0.001 to 0.05mg/kg). The concentration of arsenic in grains ranged from (0.02 to 0.50mg/kg). The concentration of cadmium in grains ranged (0.001 to 0.04 mg/kg). The concentration of copper in grains ranged (0.85 to 3.50mg/kg). The concentration of lead in grains ranged (0.003 to 0.08mg/kg). The concentration of tin in grains ranged (0.19 to 1.00mg/kg). The concentration of zinc in developed product ranged (9.23 to 15.54 mg/kg). A permissible amount of heavy metals were found in all the grains.

Table 3.2: Microbial load of standard cookies and millet based developed cookies on different days.

Parameters	Samples Days	T0	T1	T2	T3	T4	F- Value	P- Value
Total Bacterial Count (cfu/g)	7 th	210.0±1.0	200.1±1.02	227.1±1.05	224.0±1.05	231.0±1.82	3.32	0.89
	15 th	240.2±1.03	220.1±1.75	247.0±1.70	237.1±1.85	246.2±1.37	5.50	1.11
	30 th	260.2±1.00	230.2±1.04	295.0±1.0	282.2±1.01	296.0±1.1	3.25	0.99
Total Fungal Count (cfu/g)	7 th	10.2±1.00	10.0±0.55	13.1±1.25	11.2±1.00	16.2±0.85	0.00	0.001
	15 th	13.2±0.60	10.1±0.52	19.1±1.05	18.1±1.02	24.0±1.08	3.76	0.002
	30 th	15.2±0.92	10.2±0.53	25.0±1.30	29.0±1.1	33.1±1.01	4.69	0.006

T0- Standard cookies (prepared from refined wheat flour by using 100%).

T1- Experimental Cookies (prepared from pearl millet by using 80%,wheat flour-20%),

T2- Experimental Cookies (prepared from sorghum millet by using 80%, wheat flour-20%),

T3- Experimental Cookies (prepared from finger millet by using 80%, wheat flour-20%),

T4- Experimental Cookies prepared from Mixed millets by using all three millets (pearlmillet-25%, sorghum-25%, finger millet-25%) and wheat flour-25%

Data was analyzed using Anova (one way)

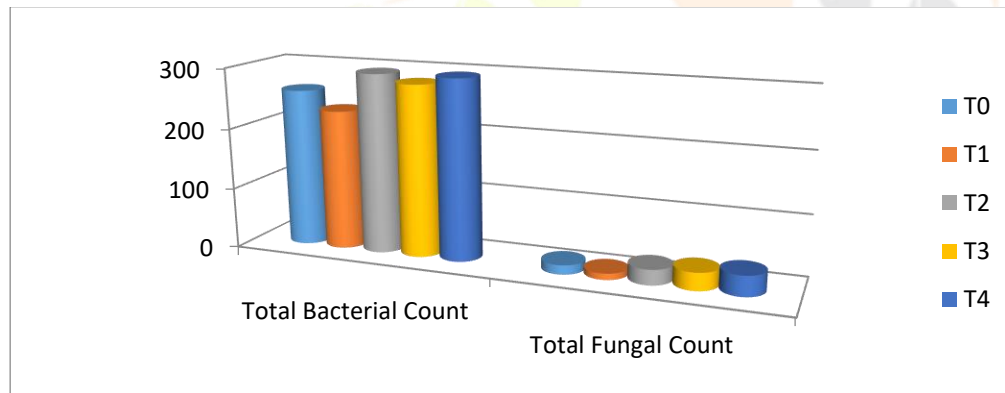
Figure:- Microbial load of standard cookies and millet based developed cookies on 30th days.

Table 3.2 depicts the microbial load of standard plate count and the acidity of product used to determine the shelf life of the product. The total plate count of T0 varied from (210 to 260cfu/g) during 30th day of storage. Total bacterial count was found in developed cookies T1, T2, T3 and T4 ranged from (200 to 230cfu/g), (227 to 295cfu/g), (224 to 282cfu/g) and (231 to 296cfu/g) respectively at 30th day of storage. The yeast and mould count of T0 varied from 10 to 15 cfu/g during 30th day of storage. Total fungal count was found in developed cookies T1, T2, T3 and T4 ranged from (10 to 10cfu/g), (13 to 25cfu/g), (11 to 29cfu/g) and (16 to 33cfu/g) respectively at 30th day of storage. The result concluded that all the variations of cookies were found safe and permissible on room temperature.

4- CONCLUSION-

The result concluded that the study was performed on product development. In this study there were two types of cookies were developed, where T0 was the standard cookies prepared with whole wheat flour and T1, T2, T3

and T4 were experimental cookies prepared with different types of millets flour (Pearl millet, Sorghum millet and finger millet). The metallic elements (zinc, copper, cadmium, tin, lead, arsenic and mercury) were analyzed in selected grains. The limits of heavy metals in grains were found in permissible amount. The recipe was standardised with 4 replications and 20 trials. After development the products were evaluated by microbial count to determine the shelf life of cookies at 30 days on room temperature. The result concluded that all the variations of cookies were found safe and permissible during storage period.

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