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Development of nutritionally enriched eggless gluten-free multigrain cookies

Vaishnavi P Jamdar, Viraj R Mohite, Virendra N Dhage, Yashodhan S Kunjir, Yudhishtir S Karad, Yajuvendra Taware, Abhilasha B Shingote*
School of Food Technology, MIT ADT University, Pune, Maharashtra, India
Corresponding Author: Abhilasha B Shingote
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Abstract
There has been an increasing tendency towards gluten-free and health-focused bakery products, leading to the use of alternate cereal and pseudo-cereal products in their formulation. One such cereal grain, sorghum (Sorghum bicolor), is free of gluten content, along with being an excellent source of dietary fiber, iron, magnesium, phosphorus, and phenolic antioxidants. The amaranth is a good source of quality protein and essential amino acids as well as a high amount of calcium and iron. Water chestnuts supply the bakery products with potassium, phosphorus, and functional starch qualities. The current research attempted to develop egg-free multigrain cookies by using sorghum as the main flour and amaranth and water chestnut flour as additives. Different composite flour combinations (T0-T5) were prepared by changing the amount of amaranth and water chestnut flour to test their impact on the sensory attributes of cookies. Jaggery was used as a natural sugar source, while roasted pumpkin seeds and chocolate pieces were added to improve the nutritional quality and sensory attributes of cookies. It was found that all formulated cookies were acceptable, but the formulation T3 possessed high acceptability with good texture, flavor, and taste. The increased sensory quality of the T3 can be credited to the optimum ratio of the composite flour used, thereby contributing to the better texture and the improved overall quality of the cookies. The developed cookies also possessed enhanced nutritional value owing to the use of highly nutritious ingredients. Hence, the developed cookies can be regarded as an effective gluten-free functional baked product.

Keywords: Gluten-free, multigrain, cookies, eggless

Introduction

In recent times, rising concerns over the link between food and health have played a vital role in shaping consumers' preferences towards certain foods. The current consumers are not only interested in the taste of the foods but are also interested in foods which offer added advantages. Consequently, there is rising consumer interest in functional foods and value-added foods which aid in improving overall health and well-being. Among the many ready-to-eat snacks, bakery products are very popular and cookies stand out as one of the most widely consumed products. Cookies enjoy great popularity among all generations and represent one of the best food products for nutritional fortification (Di Cairano *et al.*, 2020) [4]

Regular cookies are normally made with white flour from wheat, which mostly supplies carbohydrates and has lesser amounts of dietary fibers, minerals, and phytochemicals. Furthermore, foods made from wheat are not ideal for people who are intolerant to gluten and those with celiac disease. The term gluten is used to refer to a complex of proteins found in wheat, giving elasticity and structural support to baked foods. However, the intake of gluten can lead to problems such as indigestion among gluten-intolerant people (Hager & Arendt, 2013) [5].

Sorghum (*Sorghum bicolor*)

Sorghum is a significant gluten-free cereal, and it is extensively cultivated in semi-arid conditions because of its nutritional benefits. It is a source of high dietary fiber, resistant starch, iron, magnesium, phosphorous, and phenolic antioxidants. It has a lower glycemic index and hence is advantageous for diabetics and health enthusiasts (Taylor & Awika, 2017) [3]. Earlier research have shown that the addition of sorghum flour to bakery products enhances nutritional value and antioxidants, while developing products without gluten (Saleh *et al.*, 2013) [2].

Amaranth (Amaranthus)

Amaranth is a nutrient-dense pseudo-cereal grain, and it is distinguished by its excellent protein quality with balanced essential amino acids such as lysine, which is scarce in cereals. Besides, amaranth is rich in minerals like calcium, iron, dietary fiber, and bioactive compounds. Owing to its nutritional density, amaranth is utilized as a vital ingredient in gluten-free food products development. Amaranth flour addition to bakery products enhances protein quality, mineral composition, and nutrition (Kaur *et al.*, 2017) [8].

Table 1: Chemical Composition of Amaranth Grain Flour:

Chemical Component	Amaranth Grain Flour
Crude protein content (%)	14.60 ± 0.13
Moisture content (%)	4.17 ± 0.28
Crude fat content (%)	8.28 ± 1.05
Ash content (%)	1.87 ± 0.04
Total carbohydrates (%)	71.09 ± 1.32

Tanimola A. R., Otegbayo B. and Akinoso R. (2016) [17]

Water Chestnut (*Trapa natans*)

Water chestnut flour is an additional underused gluten-free flour ingredient with high nutritive and functional values. It is a good source of carbohydrates, mineral elements like potassium and phosphorus, and natural antioxidants. The use of water chestnut flour increases the ability of gluten-free flour-based products to absorb water and act as a binder. This is vital in improving the texture and structural integrity of gluten-free bakery products (Kumar & Tanwar, 2018) [6]. Incorporation of this ingredient in food products will ensure higher quality of products while enhancing the exploitation of underused agricultural crops.

In addition to flour sources, the inclusion of natural sweetening agents and ingredients with high nutritive value will increase the quality of cookies. Jaggery is considered an alternative to processed sugar since it is rich in mineral elements such as iron and calcium. Pumpkin seeds provide healthy oils, zinc, magnesium, and plant protein. Chocolate chips contribute to the taste, appearance, and sensory acceptance of the cookie product.

Although there are advantages regarding the nutrition of gluten-free raw materials, the development of gluten-free bakery products is still difficult due to the lack of gluten that affects dough extensibility, texture, and structure (Hager & Arendt, 2013) [5]. As a consequence, gluten-free cookies tend to have low texture, crispness, and acceptability (Hager & Arendt, 2013) [5]. In this regard, optimization of the flour mixture is required for balancing nutritional improvement and acceptability.

Table 2: Chemical Composition of Water Chestnut Flour

Parameters	Water Chestnut Flour
Moisture	9.36 ± 0.23
Crude Protein	8.03 ± 0.14
Crude Fat	2.33 ± 0.02
Ash	2.76 ± 0.01
Crude Fibre	0.96 ± 0.03
Carbohydrates	76.55 ± 0.10

Manisha and Dr. Darshan Punia (2020) [18]

Several pieces of literature exist on the application of different flours in bakery products, but few papers address the combination of flours made from sorghum, amaranth, and water chestnut to produce eggless gluten-free cookies. Hence, this paper aims to investigate the physicochemical, nutritional, microbiological, and sensory properties of multigrain eggless gluten-free cookies made from sorghum, amaranth, and water chestnut flour to find the most acceptable formulation.

Materials and methods

Materials

The raw materials required to produce eggless gluten-free multigrain cookies were collected from the local market at Pune, India. Sorghum flour *(Sorghum bicolor*), amaranth flour, and water chestnut flour *(Trapa natans)* were the main raw materials utilized. Other materials used were jaggery for sweetness, butter, milk, baking soda, roasted pumpkin seeds, and chocolate chips. All ingredients were of food grade quality.

Methods

Preparation of Cookies

Cookies of various formulations were prepared by varying the amount of amaranth and water chestnut flour with fixed amounts of sorghum flour. In total, six treatments (T0 to T5) were formulated.

Table 3: Standardization of cookies recipe

Sample	Sorghum (g)	Amaranth (g)	Water Chestnut (g)
T0	50	50	0
T1	50	45	5
T2	50	40	10
T3	50	35	15
T4	50	30	20
T5	50	25	25

Jaggery (40 g), butter (40 g), milk (50 ml), roasted pumpkin seeds (3 g), and chocolate chips (5 g) were kept constant for all formulations.

Preparation of Cookies

The cookies were prepared using a modified method suitable for gluten-free dough systems.

Raw Material Selection
(Sorghum flour, Amaranth flour, Water chestnut flour, jaggery, butter, milk, pumpkin seeds, chocolate chips)
↓
Weighing of Ingredients
↓
Sieving and Mixing of Dry Ingredients
↓
Preparation of Wet Mixture
(Jaggery + Butter + Milk)
↓
Blending of Dry and Wet Ingredients
↓
Addition of Pumpkin Seeds and Chocolate Chips
↓
Dough Preparation
↓
Refrigeration (15 min)
↓
Shaping and Baking (170–180°C)
↓
Cooling
↓
Packaging and Storage

Fig. Process flowchart for cookies

Fig. Cookies

Proximate Analysis

The proximate analysis of the formulated cookies was done through standard AOAC (2005) [13] procedures.

a. Moisture

The moisture content was determined using the AOAC (2005) method. The moisture content of the cookies was analyzed through the hot air oven method based on the weight loss after drying and was calculated using the formula below:

$$\text{\% Moisture} = \frac{loss\ in\ weight}{weight\ of\ sample} \text{ x } 100$$

b. Protein

The protein content was determined using the Kjeldahl technique, where nitrogen was quantified into protein using the 6.25 ratio.

Protein %= Nitrogen% x 6.25

c. Fat

The crude fat content in the samples was established by using Soxhlet apparatus through the AOAC (2005) method. Fat content was measured using Soxhlet Extraction method The percentage crude fat was calculated from the following expression:

$$\text{\% Crude fat} = \frac{Weight\ if\ Oil}{weight\ of\ sample} \text{ x } 100$$

d. Ash

Churning the sample over an electric heater and drying it at 100oC. Ash was then heated to 550 degrees Celsius for five hours in a muffle furnace. This formula was used to calculate it:

$$\text{\% Ash} = \frac{\text{Weight of Ash}}{weight\ of\ sample} \text{ x } 100$$

e. Dietary Fiber

Total dietary fibers were calculated using AOAC (2005) method using the formula,

$$\text{\% Dietary Fiber} = \frac{\text{Weight of residue} - \text{Weight of ash}}{weight\ of\ sample} \text{ x } 100$$

f. Minerals

The amounts of magnesium (Mg), zinc (Zn), and phosphorus (P) in the food samples were quantified via Atomic Absorption Spectrometry at different wavelengths of (285.2 nm), (213.9nm), and (213.6), respectively, after digestion via microwave-accelerated acid digestion method. The final amount of the element present in the food.

$$\text{Elemental content (mg/100)} = \frac{\text{C} \times \text{V} \times 100}{\text{W} \times 1000} \text{ x } 100$$

Where:

- **(C)** = Element concentration in the analysed solution (g/mL) or (mg/L) read from the AAS instrument.
- **(V)** = Total final volume of the digested sample solution (mL).
- **(W)** = Initial weight of the food sample used for digestion (g).

Spread Ratio

The spread ratio was determined by dividing the cookie's diameter by its thickness.

$$Spread\ Ratio = \frac{\text{Diameter of cookie}}{\text{Thickness of cookies}} \text{ x } 100$$

Antioxidants

Antioxidants inhibition was determined by DPPH and FRAP methods.

DPPH assesses the efficiency of your substance in donating electrons or hydrogen ions to the DPPH radical. Calculation determines the % DPPH Inhibition (scavenging ability)

$$\text{DPPH Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \text{ x } 100$$

Where,

Acontrol: Absorbance of the DPPH solution without any sample (DPPH radical + solvent)

$A_{sample:}$ Absorbance of the DPPH solution after reacting with your sample extract.

The FRAP assay determines the capacity of your sample to reduce ferric iron into its corresponding ferrous state. Ferric reducing antioxidant power (FRAP) was determined using methanolic extract prepared from about 1 g of cookie sample. Absorbance was measured at 593 nm.

$$\text{FRAP Value} = \frac{Absorbance\ of\ sample}{\text{Standard curve value}} \text{ x } 100$$

Total Plate Count

Total plate count was calculated by the regular lab media method.

Microbiological quality of the developed cookies was evaluated using total plate count, yeast and mold count, and coliform analysis. About 1 g of cookie sample was aseptically homogenized in sterile diluent and subjected to serial dilution for microbial analysis.

$$\text{CFU/g} = \frac{Number\ of\ colonies\ x\ Dilution\ factor}{\text{Weight of sample}} \text{ x } 100$$

Sugars Analysis

The reducing sugars were measured using the IS guidelines, the non-reducing sugars were measured by deducting the reducing sugars from total sugars, whereas total sugar content was calculated by adding both non-reducing and reducing sugars. Added sugars were measured according to the FSSAI guidelines.

Non-reducing sugar = Total sugar – Reducing sugar

Texture Analysis

Texture analysis was carried out using whole cookie samples. Individual cookies were analyzed using a texture analyzer to determine the maximum force required to break the sample. Hardness=Maximum force required to break cookie

Results and discussion

Sensory Evaluation

Based on the results of sensory evaluation, all formulated cookie samples were found to be acceptable by the panelists. There was an increasing trend in sensory scores for factors like colour, texture, taste, mouthfeel, and overall acceptability as T0 increased to T3. The reasons behind the improvements in sensory scores could be related to the optimum ratio of the composite flours that contributed to better taste and texture.

T3 sample had the highest overall acceptability score, meaning that there was a good trade-off between nutritional value and sensory attributes. Nevertheless, an increment in the substitution rate led to reduced sensory scores, probably because of the changes in texture and softness of the product.

Table 4: Sensory analysis

Sample	Colour	Texture	Taste	Mouthfeel	Aftertaste	Overall Acceptability
T0	8.2	8.0	7.8	7.9	7.7	7.9
T1	8.4	8.2	8.1	8.0	8.0	8.1
T2	8.6	8.5	8.4	8.3	8.2	8.4
T3	8.9	8.8	8.7	8.6	8.5	8.8
T4	8.3	7.9	8.0	7.8	7.9	8.o

The sensory evaluation of the developed cookies was done by using a 9-point hedonic scale for appearance, taste, texture, aroma, and overall acceptability. The use of water chestnut flour helped improve the sensory quality and acceptability of the cookies because of its properties, leading to better crispness and texture, thus confirming the previous studies (Gupta & Awasthi, 2021) [23].

Proximate Composition

The proximate analysis of the produced cookies revealed that there were notable differences in their values because of the alterations made in the composite flour mixture. The improved sample (T3) contained a more nutritious composition than the control one (T0).

Table 5: Proximate Composition

Parameter	T0 Value	T3 Value
Energy (kcal)	483.94	487.19
Carbohydrates	65.3%	61.4%
Fat	21.7%	23.2%
Protein	5.1%	6.5%
Dietary Fiber	3.4%	3.8%
Moisture Content	2.7%	3.1%
Ash	1.8%	2.01%

The energy value of T3 was slightly greater than that of T0. This difference reveals the rise in caloric value owing to addition of healthy foods. There was a slight reduction in carbohydrate content that could be a result of replacement of some portions of base flour with amaranth flour and water chestnut flour. On the other hand, the protein content in T3 was relatively greater than that of T0. Fat content increased in T3, which helped enhance its texture and mouth feel. There were increases in dietary fiber content, showing that there were improved functional qualities for the flour, which could be beneficial to digestion. Ash content increased in T3, as the mineral content had been increased by using composite flours.

It can thus be seen that the optimal mixture had improved nutritional value over the control sample.

Nutrition in cookies was enhanced by adding flour made from water chestnuts. Protein levels increased from 5.1% to 6.5% owing to nutritional value provided by amaranth and water chestnut flour. Fiber content rose from 3.4% to 3.8%, which can be explained by fiber and resistant starch found in water chestnut and sorghum flours. Ash levels rose from 1.8% to 2.01%, showing an improvement in mineral content as water chestnuts contain high amounts of minerals such as calcium, phosphorus, potassium, and iron. Such improvements have been noted in baked products made using composite flour. The amount of carbohydrates was reduced from 65.3% to 61.4%. This could be attributed to the substitution of carbohydrate-rich flour with flour with high amounts of fiber and proteins. The fat content increased from 21.7% to 23.2%, resulting in an increase in energy content. The moisture content increased from 2.7% to 3.1%. This could have been due to the high-water retention ability of water chestnut flour. In general, water chestnut flour improved the nutritional composition of cookies. (Singh *et al.,* 2008) [1]

The addition of water chestnut flour resulted in better nutrition and functionality of biscuits due to higher levels of protein, dietary fiber, phenolics, flavonoids, and antioxidant capacity as compared to control samples. The amount of protein was raised from 5.10% to 8.23% after flour supplementation, thereby improving the nutritional quality of biscuits. In conclusion, the use of water chestnut flour in fortified biscuits is feasible (Mohammad *et al.,* 2024) [19].

The addition of soy flour and water chestnut flour enhanced the nutritional value of cookies due to increased protein, fat, and ash content, but moisture content reduced. Sensory qualities were also enhanced up to 30% flour addition, suggesting that the use of water chestnut flour is acceptable in functional bakery products. (Sharma & Devi, 2021) [23]

Inclusion of chestnut flour resulted in the improvement in the nutrient composition of gluten-free biscuits through the increase in the amounts of proteins, lipids, fibers, ash, minerals, and reduction in the quantity of carbohydrates. Antioxidant properties were also observed to be better in chestnut flour biscuits. In the case of protein content, the control biscuits had 6.88%, which increased to 7.93% in the case of chestnut flour biscuits. Crude fiber content increased from 2.73% to 8.44%. It can be concluded that chestnut flour is appropriate to produce functional bakery products. (Stoin *et al.,* 2025) [21].

Physicochemical Properties

The physicochemical characteristics of the developed cookies indicated acceptable product quality and stability.

Table 6: Physicochemical Properties

Parameter	T0	T3
Total Phenolic Content	95 mg GAE/100 g	95 mg GAE/100 g
DPPH Activity	34%	38%
FRAP	207 µmol Fe^{2+}/100 g,	210 µmol Fe^{2+}/100 g,
Hardness	22.3 N	22.5 N

The addition of water chestnut flour enhanced the antioxidant quality of the cookies. The DPPH activity level was raised from 34% to 38% while the FRAP value was raised from 207 to 210 µmol Fe^{2+}/100 g because of the presence of active compounds and antioxidants in water chestnut flour. Nonetheless, the total phenolic content did not change, remaining 95 mg GAE/100 g. The hardness increased from 22.3 N to 22.5 N due to higher fiber content and water-binding ability.

This could be due to the presence of antioxidants in the flour of sorghum, amaranth, and water chestnut. This is because amaranth contains high levels of polyphenols and flavonoids, thus improving antioxidant activity. The total phenolic content did not change, although there was a slight increase in the hardness, which could be due to increased fiber and water retention ability. (Chauhan *et al.,* 2015) [10]

The use of soy and water chestnut flour enhanced the nutritional value of cookies by adding proteins, fats, and minerals. Acceptable sensory attributes and overall acceptability were also noted due to the use of flour. The modification in texture and hardness was due to the functional properties of the composite flour. Thus, the use of water chestnut flour is ideal for functional bakery products. (Sharma & Devi, 2021) [23],

The use of water chestnut flour contributed to the enhanced nutritional and sensory attributes of the biscuits because of its high fiber, mineral, and phenolic content. The biscuits that contained more water chestnut flour exhibited better sensory attributes. This improvement in functionality can be attributed to the ability of the water chestnut flour to absorb water and stabilize. (Gupta & Awasthi, 2021) [23]

Mineral Composition

The developed cookies contained minerals like magnesium, phosphorus, and zinc. The optimized sample had a little higher concentration of minerals than that of the control sample.

This could be due to the addition of amaranth and water chestnut flour, both of which are rich sources of minerals. Minerals are important for their contribution to metabolic and physiological processes, thus improving the nutritive value of the developed cookies.

Table 7: Minerals composition

Mineral	T0	T3
Magnesium	39 mg/100 gm	42 mg/100 gm
Phosphorus	109 mg/100 gm	110 mg/100 gm
Zinc	0.9 mg/100 gm	1.2 mg/100 gm

Addition of water chestnut flour in cookie preparation led to better mineral content. In comparison to the control sample, magnesium content was increased from 39 to 42 mg/100g, phosphorus from 109 to 110 mg/100g, and zinc content from 0.9 to 1.2 mg/100g in T3.

This increase in mineral contents could be due to the presence of abundant minerals in water chestnut flour and its ability to preserve the minerals even after baking. Such results have been observed previously in bakery products prepared using water chestnut flour (Hussain *et al*., 2020) [25].

The rise in the mineral content of T3 can be explained by the addition of flour from water chestnuts, which are naturally high in minerals like magnesium, phosphorus, zinc, calcium, and iron. This is in line with studies that found an improvement in mineral content in bakery items supplemented with chestnut flour (Zlateva *et al.,* 2024) [26].

Microbiological Analysis

The microbiological analysis showed that the cookies produced were safe for human consumption. There was an acceptable level of total plate count, and no yeast, molds, or coliform bacteria were detected. This shows that the right hygiene standards were adhered to during the process of manufacturing. The low microbial count may be due to the low moisture levels of the cookies and the high temperature at which the cookies were baked.

Table 8: Microbiological analysis

Parameter	Unit	Result	Permissible Limit*
Total Plate Count	cfu/g	$< 10^3$	$\leq 10^4$
Yeast & Mold Count	cfu/g	Not Detected	$\leq 1 \times 10^2$
Coliform	cfu/g	Absent	Absent

Analysis of microbial load in cookies revealed that the Total Plate Count was $<10^3$ cfu/g, Yeast and Mold Count was not detected, and Coliforms were absent, indicating high microbiological quality and safety of the developed cookie. These parameters were within the allowable limits according to the FSSAI guidelines for bakery and ready-to-eat food products. Microbial load can be reduced due to low moisture content, proper baking, and good manufacturing practices, which hinder the growth of microorganisms and enhance shelf life (FSSAI, 2024).

Conclusion

The current study successfully formulated nutritionally fortified eggless gluten-free multigrain cookies using sorghum, amaranth, and water chestnut flours. It was found that the use of water chestnut flour had significantly enhanced the nutritional value of cookies through increasing protein content, dietary fiber, ash content, minerals, and antioxidant capacity, while decreasing carbohydrate content. Additionally, the developed cookies had higher magnesium, phosphorus, and zinc content, which indicated better micronutrient composition. Based on sensory evaluation, it was concluded that the addition of water chestnut flour had improved the texture, crispiness, flavor, and overall acceptability of cookies. Moreover, it was found that antioxidant activity and FRAP value were also enhanced owing to the presence of antioxidants in sorghum, amaranth, and water chestnut flours. Though there was an increase in hardness, the cookies retained their desirable textural qualities. It was further determined that the developed cookies were safe for consumption through microbial testing, as the Total Plate Count, Yeast and Mold Count, and Coliform Count were found to be within the prescribed FSSAI limits. Therefore, the study has successfully indicated that water chestnut flour can be used in formulating functional gluten-free multigrain cookies with enhanced nutritional, sensory, antioxidant, and microbiological qualities.

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