"DEVELOPMENT OF SNACKS BY THE INCORPORATION OF

AMARANTH SEEDS"



DISSERTATION

Submitted in Partial Fulfillment of the Requirement for

The Award of the Degree of

MASTER'S PROGRAMME IN

CLINICAL NUTRITION AND DIETETICS

BY

NAMIYA P S

(Register No: SM19MCN009)

DEPARTMENT OF CLINICAL NUTRITION AND DIETETICS

WOMEN'S STUDY CENTRE

ST. TERESA'S COLLEGE (AUTONOMOUS)

ERNAKULAM

MARCH 2020

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Signature of Internal

Signature of External

DECLARATION

I hereby declare that the thesis entitled "DEVELOPMENT OF SNACKS BY THE INCORPORATION OF AMARANTH SEEDS" submitted in partial fulfillment of the requirement for the award of the Degree of Master's Programme in Clinical Nutrition and Dietetics is a record of original research work done by me under the supervision and guidance of Ms. Surya M Kottaram, Assistant Professor, Department of Clinical Nutrition and Dietetics, Women's Study Centre, St. Teresa's College(Autonomous), Ernakulam and that the thesis has not previously formed on the basis for the award of any degree work has not been submitted in part or full or any other degree/diploma/ fellowship or the similar titles to any candidate of any other University.

Place:

NAMIYA P S

Date:

CERTIFICATE

I hereby certify that the dissertation entitled "DEVELOPMENT OF SNACKS BY THE INCORPORATION OF AMARANTH SEEDS" submitted in partial fulfillment of the requirement for the award of the Degree of Master'sProgramme in Clinical Nutrition and Dietetics is a record of original research work done by Ms. NAMIYA P S during the period of her study under my guidance and supervision.

Signature of the HOD

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ABSTRACT

DEVELOPMENT OF SNACKS BY THE INCORPORATION OF AMARANTH SEEDS

Amaranth, is an ancient crop which is used as a high-protein seed or as leafy vegetables. Grain amaranth species are important in several parts of country and at different times for several thousand years. Grain amaranth is drought-tolerant, provided there is sufficient moisture to establish the crop. Amaranth responds well to high sunlight and warm temperature . One of the main reason there has been recent interest in amaranth is due to its useful nutritional qualities. The grain has 12 to 17% protein, and is high in lysine, an important amino acid by which cereal crops are low. The grain is high in fiber and low in saturated fats, these factors contribute to its use by the health food markets. The aim of the study was to develop snacks by incorporating amaranth. Amaranth cookies, Amaranth Laddoo, Amaranth Spicy Sevai and Amaranth snack bar are the four different snacks made with amaranth. Three variation of developed products were made. The organoleptic analysis of the variation was undertaken by untrained panel who analyzed the taste, color, texture, appearance, and overall acceptability. Based on the score of nine hedonic rating scale, the most accepted among the three variations was identified. Shelf-life analysis was done comparing the fresh amaranth seed flour and flour which was kept for three months duration. Nutrient content analysis of the selected variation was conducted. The tests included protein estimation by Kjeldhal method, Calcium estimation by EDTA method, Phosphorus estimation by Colorimetricmethod, Iron by phenanthroline method and dietary fiber by enzymatic gravimetric method. Packaging and labeling of the products were also done.

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INTRODUCTION

1. INTRODUCTION

Amaranthus belonging to the Amaranthaceae comprises a series of untamed or wild, weedy, and cultivated species and located worldwide in most agricultural environments. Amaranthus species have different centers of domestication and origin, being wildly distributed in North America, Central America, and South American Andes where the best genetic diversity is found. Among all species Amaranthus caudatus, Amaranthus hypochondriacus and Amaranthus cruentus are mainly cultivated for their seeds (Rastogi and Shukla, 2013) (Bressani 2003) and are considered as pseudocereals with a good amount of seed protein content and a high lysine content.

The word Amaranthus is essentially derived from the Greek word "anthos" (flower) which suggests everlasting or unwilting. Popularity within the cultivation and consumption of Amaranthus seed within the era began within the mid-1970s with the rediscovery and promotion of amaranth is mainly because of its superior nutritional attributes as compared to cereal grain. Amaranth is considered as a "superfood" because it contains high nutraceutical values like a high-quality protein, unsaturated oils, squalene, dietary fiber, tocopherols, tocotrienols, phenolic compounds, flavonoids, vitamins, and minerals. Compared to other grains, amaranth features a higher amount of protein, dietary fiber, calcium, iron, and magnesium; therefore, although it's an ancient crop nowadays, it's considered a millennium crop or superfood with relevant nutraceutical values and its agronomic versatility (Manuel and Isabel, 2019)

Amaranth is protectant against heat and drought with no major disease problems (Robert et al 2008, Bario and Anon, 2010). It is a rare plant whose leaves are eaten as vegetable

while seeds are eaten as cereals. Because of its similarity with other cereals, it can be considered as a pseudo-cereal which may be alternative rich source of protein and nutrient for poor people in developing countries.

Amaranth may be a plant with high nutritional value, whose nutrients are concentrated within the leaves and therefore the grains. Amaranth seeds have attracted attention as a person's nutritional source because they contain higher amount of protein with a well-balanced amino acid composition also as minerals, vitamins, and phytochemicals compared to those of major cereals like wheat and rice (Alvarez et al 2010).

The seeds contain high amounts of dietary fiber, iron, and calcium. They even have high amounts of lysine, methionine and cysteine, combined with a fine balance of amino acids, making them a wonderful source of top quality, balanced protein, which is more complete than the protein found in most grains. It is also characterized by high lipid content than most cereals and contains between 50 and 60 g of starch per 100g of grains (Alvarez- Jubeteet al, 2010). In additional to its outstanding nutritional value, amaranth is also very low in sodium and contains no saturated fat.

Amaranth grain consists of proteins (13–22%), lipids (5–13%), dietary fiber (9–14%), vitamins (ascorbic acid, riboflavin, niacin), minerals, and other phytoconstituents including betalains(Karama'c&Miguel 2018)

The main element is linolic acid which the human organism is not ready to produce, and which is important for its existence. Amaranth grain contains tocotrienols and squalene compounds, which are known to affect cholesterol biosynthesis (Rodas&Bressani 2009).

Squalene may be a substance of the isoprenoid type which is a precursor within the synthesis of steroids and important anti-oxidizing substances such as vitamin Q 10 (ubiqui-none). Squalene itself has favourable anti-oxidizing properties – thus it protects several body structures against oxidative damage (Reddy &Couvreur 2009; Waterman & Lockwood2007).

The amaranth grain at household level are often cooked and eaten as porridge (Mugalavai 2013), it also can be malted for beer production, it's been demonstrated that grain amaranth are often popped or roasted for flour production which may be used singly or mixed with other food ingredients to form products like bread, biscuits, pasta, crackers, pancakes, muffins, paste and breakfast cereal (Emire and Arega 2012) In addition to nutritive value, amaranth seed has various health benefits and medicinal properties including gastric problems, blood purification, regular consumption reduces blood pressure and cholesterol levels and improving antioxidant status.

In Mexico, the popped amaranth confection, 'alegria' is a popular favourite among locals and tourists. The flour or flaked forms are mixed with wheat or other flours to make bread, cookies and other baked goods. Coarsely ground amaranth is employed to form a tasty and nutritious porridge cooked by itself or mixed with other grains and pseudo cereals like oats, wheat, milled flax seed.

The grain amaranth may be a promising underutilized food crop because it can grow during a wide selection of weather. It is an excellent drought tolerant crop with inherent strong market and industrial potentials which are yet to be fully tapped (Olaniyi 2007; Akin-Idowu et al. 2017). Amaranth has the power to grow and adapt in extremely harsh weather (Olufolaji, Odeleye, and Ojo 2010). It can be successfully cultivated for leaf or grain in many regions and seasons where other crops cannot thrive (Mlakar et al. 2009)

The most frequent mode of consumption of amaranth grains is after popping and milling and by mixing the flour with other cereal flours such as teff, sorghum, barley, and wheat to prepare bread, injera and porridge. (SanzPanella, 2012).

SouthAmericans parch or cook it for a gruel or porridge, or mill it to produce light coloredflavor.As a snack the grain is popped and tastes like nutty flavoredpopcorn.

According to Ljubica et al, (2009) he reported that amaranth flour can be used to partially replace regular corn flour for extruded snack manufacturing. The mixture of instant whole amaranth and rice can be used to produce extruded flours to be used in formulations of beverages (Xaene et al, 2008) and extruded snacks can be manufactured from defatted amaranth flours. (Rosa et al, 2015)

Children, high-geared athletes, gluten and lactose intolerant people, diabetic individuals, and coeliacs can have amaranth products concerning nutritional aspects. (Valcárcel-Yamani,Lannes 2012)

SIGNIFICANCE

Amaranth grain is an ingredient of great interest due to its good nutritional composition, especially pale brown amaranth seed when compared with blackseeds due its very high content of vitamins and minerals. It can grow and adapt in our environmental conditions as well. The unique combination of Calcium, Iron, phosphorus, and protein can be used to supplement food products for children, and pregnant women, in order to compensate nutritional deficiencies resulting from special diets or high requirements. Also, the balanced amount of essential amino acids contributes to its overall nutritional quality. Introducing amaranth into dishes makes the diet more nutritious. Amaranth is a suitable raw material for non-leavened foods like snacks. So highlighting the benefits of amaranth and developing products using amaranth seed was found to be relevant.

OBJECTIVE:

The present study was planned with the following objectives:

- 1. To determine the shelf life of amaranth seed flour
- 2. To formulate snacks using amaranth seed flour
- 3. To conduct organoleptic analysis of developed product
- 4. To analyze selected nutrient content of developed products
- 5. To pack and label the developed snacks.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The review of literature pertaining to the study entitled "DEVELOPMENT OF SNACKS BY THEINCORPORATION OF AMARANTH SEEDS" are discussed under the following headings:

2.1 Chemical composition and nutritive value

2.2 Bioactive Components and Medicinal Properties

2.3 Health benefits of amaranth seed

2.4 Antioxidant property of amaranth seed

2.5 Antinutrient composition of amaranth seed

2.1 Chemical composition and nutritive value

The small seeds usually are shiny black in color, in comparison to those of the grain types which are cream-colored. There are up to 3000 seeds/gram. The small, lens shaped seeds are usually pale in colour. A seed of grain amaranth is on the average composed of 13.2 to 21.0% of crude protein; 5.5 to 10.9 % of crude fat; 48.0 to 69% of starch; 3.1 to 5.0% of dietary fiber and 2.5 to 4.4 % of ash (Grobelnik, 2009)

Amaranth is a pseudo cereal which is an unconventional and interesting source of proteins. Its seeds contain a large amount of highly nutritional quality proteins (Bolontrade, 2016) whose amino acid compositions are rich in lysine and methionine, the two limiting amino acids in cereals and legumes, respectively (Januszeweska Jozwiyak2008)

Amaranth's balanced amino acid composition is very close to the optimum protein reference pattern in the human diet according to FAO/ WHO requirements.

The protein is high in amino acid lysine, but it is low in leucine. This is the opposite of most other grains. Thus, proper mixing would form an almost perfect protein (Good ratio of unsaturated fat:saturated fat that is beneficial for hypertension and coronary heart disease). The nutritional quality of amaranth seed is high because of its high protein content and balanced essential amino acid composition (Ozwald2016)

Leucine, isoleucine, valine, the limiting amino acids in amaranth, are not considered a significant problem since they are found in excess in commonest grains, and thus, amaranth is well suited for blending with cereals. The main protein fractions present within the amaranth grain are albumins, 11S-globulin, P-globulin, and glutelin's.

Amaranth has recently become a focus of interest for its high nutritive values and has great potential as a functional food for its cholesterol-lowering effect observed in animal models (Mendonça2009)

It is reported that amaranth oil has high levels of tocotrienols and squalene, which are natural organic compounds that are involved in metabolism of cholesterol which could play an important role in lowering LDL-cholesterol in blood. Amaranth's lipid is exclusive with high squalene content starting from 2.4 to 8.0% of the overall oil contents (Rodas and Bressani,2009)

2.2 Bioactive Components and Medicinal Properties

The health benefits of amaranths have always been known in homoeopathic and Ayurvedic medicines. Both the seeds and leaves of amaranth are used for herbal remedies and it have nutraceutical value. Amaranth protein contains a coffee proportion of prolamins which makes it a secure ingredient for people with disorder and up so far studies have shown that amaranth peptides displayed antihypertensive and antiinflammatory activity. Peptides present in amaranth seed proteins have shown various biological activities. Some studies using amaranth flour and protein isolates reported the occurrence of peptides with biological activities like anti-hypertensive, antioxidant, antithrombotic, anti-proliferative, among others. Amaranth is ranked together of the highest five vegetables in antioxidant capacities (Walter& Das S 2016)

It contains ample number of bioactive components, like L-ascorbic acid, beta carotene, polyphenol, anthocyanins, and lutein.

The beneficial antioxidant activities for health are related to their bioactive components. The cholesterol- lowering effects in amaranth is due to unsaturated fatty acids. Being an upscale source of magnesium, which is effective to relax blood vessels and stop constriction and rebound dilation, it helps to fight migraines. Cooking processes have no deleterious effect on the total bioactive component aside from the reduction of anthocyanins content. Home cooking increases the antioxidant activities and thus the contents of arytenoids, especially by steaming. Both simmering and blanching increased the beta carotene and lutein within the cooked amaranth (Han and Xu B 2014)

2.3 Health benefits of amaranth seed

The protein present in amaranth is of an unusually high quality due to its outstanding balance and high content of essential amino acids the essential amino acids present in grain of amaranth are ideal consistent with the planet Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO). For instance, the quantity of lysine and tryptophan present in amaranth grain are relatively above those found in wheat, rice, and maize grains, but it is deficient in leucine.

Protein is used in almost every single cell in our bodies and is important for building muscle mass, supporting neurological function, aiding in digestion, helping to balance hormones naturally, and keeping an upbeat mood which suggests that this protein is beneficial for muscle recovery and therefore the system for athletic performance (Giardina S, Marzani B 2008)

Amaranthus species are ancient crops with good nutritional and therapeutic value but their full potentials are yet to be optimally exploited. Many underutilized leafy vegetables of African origin are employed by local communities in several a part of African nations for diverse therapeutic purposes (Kwenin, Wolli, and Dzomeku 2011)

The ability of those vegetables to market health benefits is essentially due to nutritional and bioactive compounds available in them (Cornejo et al. 2019). Amaranthus are highly nutritious; both the grain and leaves are utilized for human also as for animal feed (Mustafa, Seguin, and Gelinas 2011).

Moreover, it's a balanced content of essential amino acids and unsaturated fatty acids. Its protein is rich in lysine, a limiting amino acid that is absent in many other grains. (Amare et al. 2015). Amaranth was declared together of the longer-term promising crops to feed the worldwide population (Mekonnen et al. 2018). Among the green leafy vegetables and the cereals, amaranth species are considered as store house for important vitamins such as vitamin C, B6, folate and carotene (Musa et al. 2011).

The intake of amaranth products could help to avoid diseases caused by inflammations because it has been described that extruded amaranth protein hydrolysates had prevented inflammation by the activation of bioactive peptides that reduced the expression of several pro-inflammatory markers(Montoya-Rodríguez A 2014) That is the reason why consumption of amaranth grain would help to reduce inflammation.(Laparra JM 2016) it is recommended to include amaranth grain within the diet so as to scale back inflammation and should help to stop chronic diseases derived from inflammation process.

Calcium is a key player in the generation and maintenance of healthy bones as it supports mineralization (Macdonald HM 2004). Amaranth contains more calcium than other seeds, which makes it a valuable food that helps to possess a healthy development of bones helping to stop osteoporosis [Levis S, Lagari VS 2012]. Therefore, the intake of extruded amaranth products could help to enhance the right intake of calcium to support healthier bones (Galan MG,2013)

It is documented that various plant-originated "gastroprotectors" with different compositions are utilized in clinical and folk medicine because of their beneficial effects

on the mucosa of alimentary canal.Ethanolic and ethyl-acetate leaf extracts of A. tricolor showed that gastric-ulcer healing effect in acetic acid-induced chronic gastric ulcers and gastric cyto protective effect in ethanol and indomethacin-induced gastric ulcers in pylorus-ligated rats (Devaraj VC 2011) A combined use of this extract with two other herbs will help to enhance the antiulcer properties Krishna BG. (2013)

The importance of amaranth species seems enormous which make its exploration health purposes and industrial applications most imperative. Studies have shown that regular consumption of amaranths has the potential (Karamać et al., 2019) to scale back cholesterol level, benefit people affected by hypertension and disorder (Olaniyi 2007; Kolawole and Sarah 2009).

Hepatotoxicity is caused by toxic drugs, more than alcohol consumption, infection, or autoimmune response. Oral administration of methanolic extract of A. spinosus significantly increased the protein and glycogen contents in liver of Sprague Dawley rats thus indicating it to be safe for treatment of liver problems (T.Gul, K.Singh 2011)

According to Escudero et al. their observations based on the lipid profile of liver in rats, concluded that presence of phenols in flour and protein concentrate of A. cruentus seeds provokes a rise in antioxidant defenses, thus playing a protective role in liver.

The A. hypochondriacus seed of amaranth had significantly increased the activity and gene expression of Cu, Zn-SOD; decreased activity of aspartate aminotransferase (SGOT) and content of malondialdehyde (MDA) (p < 0.001) in serum; decreased NADPH oxidase transcript levels (p < 0.05) in liver, suggesting the protective effects in rats intoxicated with ethanol (V.R Lopez, G.S Razzeto, 2011)

Gandhi et al. (2011) had tested the antiproliferative activity of the species A. cruentus aqueous extract on human peripheral lymphocytes and suggested that it might be used as a cheap, biocompatible, commercial alternate to available anti-proliferative therapeutics.

According to Tagwira et al. (2006). He reported that consumption of amaranth species was found to impact health benefits among local communities. The communities claimed that eating grain amaranth made them healthier and that they also noticed significant improvements in their children's health like improvement in appetite, fast healing of mouth sores and reduction in overweight. It was also noticed that the consumption of amaranth seed led to greater production of milk among nursing mothers; this experience was seen as a positive contribution to food and nutrition security (Tagwira et al. 2006).

Amaranthus grain has been found to supply alternative food ingredient within the development of food products aside from wheat and other cereals for celiac patients. A diet is that the only therapeutic treatment currently available for patients with celiac disease; an autoimmune disease of the tiny intestine related to a permanent intolerance to gluten proteins (Martínez-Villaluenga et al., 2020). Amaranth proteins are consisted mainly of the albumin and globulin, where prolamins, the main toxic proteins for celiac patients, are very scarce.

The ability of amaranth to exhibit the health benefit is due to its bioactive compounds present in it. Bioactive compounds in amaranth grain are phenolic acids such as protocatechuic, hydroxybenzoic, caffeine, and ferulic acid, rutine, nicotiflorin and isoquercetin. There is evidence that routine slows down the process of aging, quercetin prevents oxidation, and nictoflorin helps in the protection of memory related functions (Alvarez et al. 2010).

2.4 Antioxidant property of amaranth seed

The potential of antioxidant activity of amaranth has been attributed to the presence of appreciable amount of phenolics and flavonoids. Leaves and flowers of Amaranthus also as their extracts were shown to possess highest antioxidant activities compared to other parts; rutin being the main radical-scavenger (P. Kraujalis, P.R. Venskutonis2013)

The Total antioxidant activity assay of dry amarath leaf powder of A. tricolor revealed 1 g like 0.035 g/ml of ascorbic acid (A.C. Clemente, P. V Desai2011) M.J. Iqbal,S.Hanif Z Mahmood 2012)

Evaluation of the pure and aqueous and methanolic leaf and seed extracts from A. viridis ; ethyl acetate leaf extracts of A. spinosus , revealed that they possess good radical scavenging activity confirming their excellent antioxidant activity (M.J. Iqbal,S.Hanif Z Mahmood 2012)

A study based on hydro acetonic, methanolic and aqueous extracts made from the aerial parts of A. cruentus and A. hybridus; conducted by Nana et al. described these extracts are having antioxidant and xanthine oxidase inhibitory activities in vitro (F.W.Nana, A hilou, 2012)

All plant species posess their own antioxidant defense mechanisms, which are based mainly on the chemical properties of various secondary metabolites, particularly the polyphenol compounds. Amaranth seeds are source of antioxidatively important phenolic compounds, particularly in those arid zones where commercial crops cannot be grown (Barba de la Rosa and others 2009). Antioxidant potential of amaranth seeds and leafy parts was studied using various well-known in vitro methods and new assays (Jung 2006).

The vitamins present in Amaranthusalongside carotenoids, flavonoids, and phenolic acids contribute to their high antioxidant activity, whereas squalene and tocols are important lipophilic antioxidants present mainly in amaranth grain. The antioxidant activity of different varieties of A. caudatus does not differ significantly from each other; IC50 values of ester extracts were 0.50 and 0.62 mg/mL, respectively (Conforti et al. 2005).

The methanolic crude extracts of A. caudatus were screened for DPPH° scavenging, using ascorbic acid as a standard antioxidant for comparison. The IC50 value of the methanolic extract was 0.14 mg/L; it contained 03.86 ± 0.20 mg vitamin C, 15.33 mg carotenoids, and 343.0 mg total phenols in 100 g (Veeru and others 2009). The total antioxidant capacity within the extracts of methanol of 8 edible leafy vegetables from Ghana, including A. cruentus, was lower than in the hydro-ethanol extracts (Morrison and Twumasi 2010)

Red beetroot (B. vulgaris) is the only commercial betalain source [Strack D,2003], although amaranths have also been proposed as commercial sources of betalains [Cai Y, Sun M 2003].

These betalains have some limited use in food coloring and also have several reported health benefits [Gengatharan A 2015]. Chemical and biological related experiments have demonstrated the capacity of antioxidants activity of betalains [Borkowski T 2005].

Cai et al. 2003 showed that the betalains from Amaranthaceae were stronger antioxidants than ascorbic acid, rutin, and catechins. Betalains have reported to posess anticancer properties.

Pigment extract from beetroot pomace also inhibited the growth of gram-negative bacteria and induced zones of reduced growth in Salmonella typhimurium and Bacillus cereus. Thus, amaranth plant is enriched with both nutritional and nutraceutical properties and may be a wholesome crop for the longer-term generation [Čanadanović-Brunet JM 2011]

2.5 Antinutrient composition of amaranth seed

It is reported that some of the antinutrients present in amaranth are phytate, saponins, tannins, oxalates, protease inhibitors and nitrates. The consequences of oxalates and phytates on nutrient inhibition are of more concern in amaranth grain. Tannins affect nutritive value of food by forming network with protein (both substrate and enzyme) thereby inhibiting digestion and absorption (Chemeda, A. S., &Bussa, N. F. 2018).

These antinutritional elements serve protective effects in plants, as an example, phytic acid is a sort of storage for phosphorus within the plants. But in humans, they inhibit nutrient absorption. Phytate isn't digestible by humans and is therefore not a dietary source of inositol or phosphate. Rather, it forms networks with proteins thereby limiting its availability. additionally, inhibition of starch digestion has been reported for phytate and oxalate. Saponins form complexes with mineral elements like zinc and iron; oxalate binds calcium and thus reduces its absorption (Cuadrado et al. 2019).

Different processing methods are accustomed lower antinutrient contents of amaranth. Tannins are present in high concentrations within the grain hull, removal of the hull eliminates most of the tannins, but this doesn't remove the phytic acid. Phytic acid is distributed homogenously within the grain, thus it can't be reduced by removal of the external grain layers or by water extraction.

Babatunde and Gbadamosi (2017) reported the consequences of 5 processing methods such as defatting, blanching, germination, fermentation and autoclaving on the protein digestibility and antinutrient composition of the grains Amaranthus viridis. It had been reported that germination at 30 °C for 72 hours has the foremost significant effect on protein digestibility; with 82% increase compared to fermentation which improved protein digestibility by 76%. Heat treatment like autoclaving and blanching were simpler in reducing tannin and oxalate content.

Njoki (2015) examined the results of dry heating processes and wet heating techniques and boiling amaranth flour on important parameters of grains of tumbleweed. Boiling of flour and whole grain increased the protein digestibility by 24% and 15% respectively while roasting decreased the protein digestibility. Effect of boiling in reducing tannin, phytate and oxalate content was above roasting and popping.

Kanensi et al. (2011) optimized the steeping and germination time for amaranth grain; steeping amaranth grain for five hours and germinating for twenty-four hours were recorded because the optimum processing times supported dry matter loss and reduction in antinutrient levels. Certainly, the presence of anti-nutrients in amaranth grains may

possess challenges in its utilization for food security; nevertheless, proper processing methods can minimize their antinutrient content.

METHODOLOGY

3. METHODOLOGY

The methodology of the present study entitled "DEVELOPMENT OF SNACKS BY THE INCORPORATION OF AMARANTH SEED" is discussed below:

3.1DRYING AND POWDERING OF AMARANTH SEEDS

3.2SHELF-LIFE ESTIMATION OF POWDERED AMARANTH SEED

3.3. DEVELOPMENT OF THE PRODUCTS

3.3.1 PRELIMINARY CHECKING OF AMARANTH SEEDS AND INGREDIENTS OF FOOD PRODUCTS

3.3.2 METHOD OF PREPARATION

3.4 ORGANLEPTIC ANALYSIS OF THE DEVELOPED PRODUCTS

3.4.1 SCORE CARD

3.5 ANALYSIS OF NUTRIENT CONTENT IN THE DEVELOPED PRODUCTS

3.5.1 PHYSIOCHEMICAL ANALYSIS OF PROTEIN, DIETARY FIBER, CALCIUM,

PHOSPHORUS AND IRON

3.6 PACKAGING AND LABELLING OF DEVELOPED SNACK

3.1DRYING AND POWDERING OF AMARANTH SEEDS

Amaranth grains were cleaned and washed under tap water to remove dirt dust and foreign materials. The washed grains were spread over filter paper sheet and dried completely. After drying, the grains were ground in an electric grinder to fine powder.

3.2SHELF-LIFE ESTIMATION OF THE AMARANTH SEED POWDER

Amaranth seed powder was stored in airtight glass containers at room temperaturefor estimating the shelf life. For a period of three months, amaranth seed powder was tested each month for their change in color, taste, and odor. The change in moisture, Aerobic plate count and yeast and mold growth were assessed clinically for fresh sample and the sample after 3 months.

3.3 DEVELOPMENT OF THE PRODUCTS

The study developed the products that are easy to prepare and nutritious, enhancing, and harnessing the health benefits provided by Amaranth seed. Four products were made based on standardized recipes. 3 variations of each product were made, and it was done changing the proportion of Amaranthus.

3.3.1 PRELIMINARY CHECKING OF AMARANTH SEEDS AND INGREDIENTS OF FOOD PRODUCTS

Before preparation, the organoleptic properties of amaranth seed were checked such as condition of seed, expiry date of other ingredients used. All the ingredients were measured using a weighing balance. Handling and preparation of foods were done under hygienic conditions with minimum wastage of food materials.

3.3.2 METHOD OF PREPARATION

3.3.2.1 AMARANTH COOKIES

INGREDIENTS

- Amaranth flour- 50 g
- Wheat flour-50g
- Sugar- 25 g
- Milk- 15 ml
- Cinnamon powder-5g
- Watermelon seed-5g
- Almond-5g
- Ghee-5g



PLATE 1

PREPARATION

- In a large bowl, add ghee and powdered sugar and mix it well.
- Add amaranth flour, wheat flour, powdered watermelon seeds and a

little of cinnamon powder to it

- Add milk and mix well to form dough.
- Flatten it into small disc in a baking tray and add crushed almonds to the top of it.
- Bake at 180°C for 10-15 minutes.

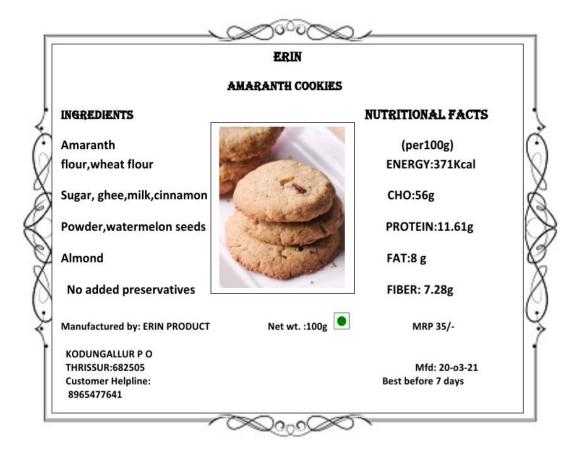


PLATE - 2

LABEL DEVELOPED FOR AMARANTH COOKIES



PLATE-3

PACKED AMARANTH COOKIES

3.3.2.2 AMARANTH LADDOO

INGREDIENTS

- Amaranth flour-30g
- Bengal gram flour-35g
- Wheat flour-35g
- Sugar- 40g
- Ghee 50g



PLATE-4

PREPARATION

- Amaranth flour, Wheat flour and Bengal gram flour were sieved separately.
- The flours were roasted separately till light brown.
- Roasted flours were mixed and fried in ghee for 2-3 minutes.
- Remove from fire and allow to cool.
- Ground sugar was added and mixed well.
- Laddoo were made of even size



PLATE- 5

LABEL DEVELOPED FOR AMARANTH LADDOO



PLATE- 6

PACKED AMARANTH LADDOO

3.3.2.3 AMARANTH SPICY SEVAI

INGREDIENTS

- Amaranth flour-40g
- Bengal gram flour-60g
- Red chili powder- 5g
- Garam masala powder- 2g
- Salt- 1 tbs
- Oil-15 ml



PLATE- 7

PREPARATION

- Amaranth flour and Bengal gram flour were mixed and sieved.
- Red chili powder, salt and a table spoon oil were added in mixture of flour.
- Stiff dough was made by using water.
- Dough was put in sev making machine and rotate it to make a desired shape.
- It is then fried in oil till golden brown.

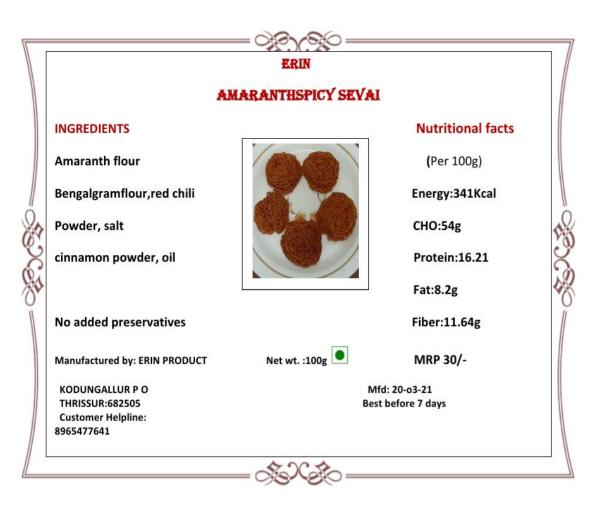


PLATE-8

LABEL DEVELOPED FOR SPICY SEVAI



PLATE-9

PACKED SPICY SEVAI

3.3.2.4 AMARANTH SNACK BAR

INGREDIENTS

- Amaranth flour-40g
- Oats-20g
- Jaggery-15g
- Sunflower seed-5g
- Pumpkin seed-5g
- Almond- 5g
- Cashew nut- 5g
- Raisins-5g



PLATE- 10

PREPARATION

- Heat a wide pan on high flame until the pan is hot.
- Add amaranth seed at that time. Cover it with a lid.
- Quickly jiggle the pan above the burner
- Transfer the puffed seeds to bowl.
- In another saucepan keep jaggery and add water to it
- Stir until it melts completely to form thick.
- After it becomes a thick paste oat, crushed sunflower seeds, pumpkinseeds, and Cashew was added along with puffed amaranth.
- Mix until it comes together in a low flame.
- Transfer to a square pan
- Smoothen it with a flat-bottomed greased bowl.
- Finally garnish it with almonds and raisins
- Press them tight and keep in refrigerator for the product to set.

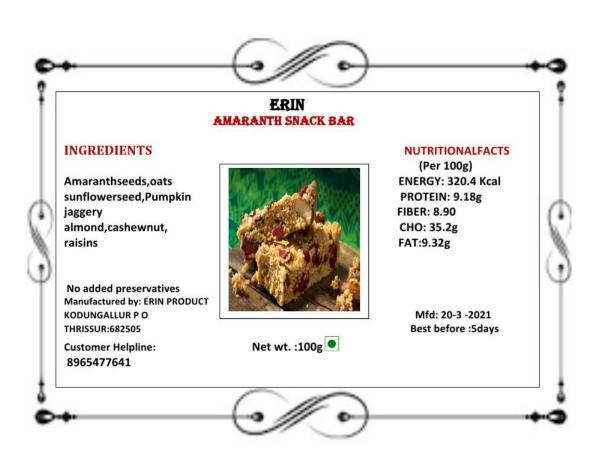


PLATE-11

LABEL DEVELOPED FOR AMARANTH SNACK BAR



PLATE- 12

PACKED AMARANTH BAR

3.4ORGANLEPTIC ANALYSIS OF THE DEVELOPED PRODUCTS

- The sensory evaluation was carried out by 10 untrained panel members.
- Samples prepared were arranged in row.



PLATE 13

• After analysis they were asked to score the samples using scorecards given to them.

3.4.1 SCORE CARD

A score card was given to the panel to rate and record the acceptability of food products based on 5 attributes such as appearance, color, texture, taste and over all acceptability. Scoring was done using 9-point hedonic scale rating system.

The sensory evaluation was carried out by 10 untrained panel members to evaluate the product. The panel were provided with cookie,laddu,protein bar, and spicy sevai of

different variations along with a score card of 9-point hedonic scale to rate the products. The card implies the degree of liking based on the numerical choices. Score 1 to 9 implied dislike extremely, dislike very much, dislike moderately, dislike slightly, neither like nor dislike, like slightly, like moderately, like very much and like extremely respectively.

According to the Institute of Food Technologists (IFT), sensory evaluation is a scientific method used to evoke, measure, analyze and interpret those responses to products as perceived through the senses of sight, touch, smell and taste (IFT 2007).



PLATE 14

Cookies sample for organoleptic evaluation



PLATE 15

Laddoo samples for organoleptic evaluation



PLATE 16

Spicy sevai samples for organoleptic evaluation





Snack bar samples for organoleptic evaluation

3.5 ANALYSIS OF NUTRIENT CONTENT IN THE DEVELOPED PRODUCTS

The variation of each product selected for the analysis was the top ranked variation in its overall acceptability which was done using the hedonic score card by the panel. Physicochemical analysis was done for evaluating the nutrient content of the developed products. Protein, Iron, calcium, dietary fiber and phosphorus content was clinically assessed.

3.5.1PHYSICOCHEMICAL ANALYSIS OF PROTEIN, CALCIUM, PHOSPHORUS, DIETARY FIBER AND IRON

The nutrient content analysis of the developed product was conducted at Neogen Foods laboratory located in Poonithura of Ernakulam District. The test included protein estimation by Kjeldhal method, calcium estimation by EDTA method, Phosphorus estimation by Colorimetric method, Iron estimation by Phenanthroline method and dietary estimation by Enzymatic gravimetric method.

RESULT AND DISCUSSION

4. RESULT AND DISCUSSION

The results and discussion pertaining to the study entitled **"Development of snacks by the incorporation of amaranth seeds**" is discussed under the following headings:

4.1SHELF-LIFE ANALYSIS OF FRESH AMARANTH SEED POWDER 4.2SHELF-LIFE ANALYSIS OF AMARANTH SEED POWDER AFTER3 MONTHS

4.3 COMPARISON OF PARAMETERS BETWEEN FRESH AMARANTH SEED POWDER AND AMARANTH SEED POWDER AFTER 3 MONTHS

4.4 AVERAGE MEAN SENSORY SCORES OF DEVELOPED PRODUCTS

4.4.1 AMARANTH COOKIES

4.4.2 AMARANTH LADDOO

4.4.3 AMARANTH SPICY SEVAI

4.4.4 AMARANTH BAR

4.5 PHYSICOCHEMICAL ANALYSIS OF SELECTED NUTRIENTS OF DEVELOPEDPRODUCTS

4.5.1 AMARANTH COOKIES

4.5.2 AMARANTH LADDOO

4.5.3 AMARANTH SPICY SEVAI

4.5.4 AMARANTH SNACK BAR

4.6 FRIEDMAN'S TEST FOR OVERALL ACCEPTABILITY OF DEVELOPED PRODUCTS

4.6.1 AMARANTH COOKIES

4.6.2 AMARANTH LADDOO

4.6.3 AMARANTH SPICY SEVAI

4.6.4 AMARANTH BAR

4.1SHELF-LIFE ANALYSIS OF FRESH AMARANTH SEED POWDER

TABLE 1

SI.No	Parameters	Amaranth seed powder
1	Moisture	4.99
2	Aerobic plate count	460
3	Yeast &mould	<100

4.2 SHELF-LIFE ANALYSIS OF AMARANTH SEED POWDER AFTER 3 MONTHS

SI.No	Parameters	Amaranth seed powder
1	Moisture	13.27
2	Aerobic plate count	24000
3	Yeast &mould	400

TABLE 2

While analyzing the shelf life of fresh amaranth seed powder it was found that the moisture content and Aerobic plate count was 4.99and 460 respectively. Yeast and mould rate is less than 100 which indicates that the product is suitable for consumption.

While analyzing the shelf life of amaranth seed powder kept for 3 months it was found that the moisture content and Aerobic plate count was 13.27and 24000, respectively. Yeast and mould rate is above 100 which indicates the product cannot be kept for 3 months as it becomes contaminated. Proper heat treatment can overcome this for the consumption.

4.3 COMPARISON OF PARAMETERS BETWEEN FRESH AMARANTH SEED POWDER AND AMARANTH SEED POWDER AFTER 3 MONTHS

SI. No	Parameters	Fresh Amaranth	Amaranth seed
		seed powder	powder after 3
			months
1	Moisture	4.99	13.27
2	Aerobic plate count	460	24000
3	Yeast &mould	<100	400

TABLE 3

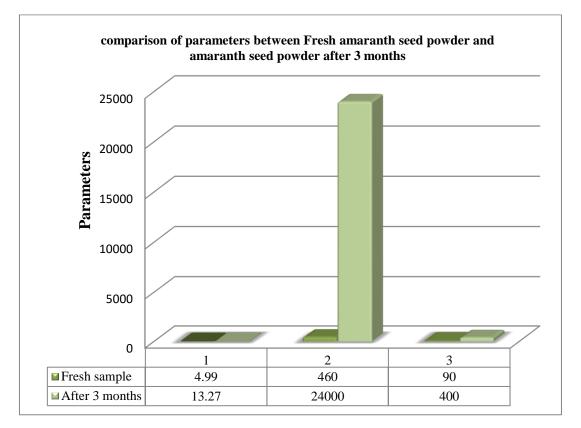


FIGURE-1

It has become evident from the figure that Amaranth flour after 3 months has higher aerobic plate count compared to the fresh flour. The Aerobic Plate Count is used as an indicator of bacterial populations. It is not actually a measure of the entire bacterial population, but it is a generic test for organisms that grow aerobically at mesophilic temperatures (25 to 40°C). Studies had shown that proper processing and cooking methods can destroy these microorganisms.

4.4AVERAGE MEAN SENSORY SCORES OF DEVELOPED PRODUCTS 4.4.1 AMARANTH COOKIES

Sensory	Appearance	Taste	Colour	Texture	Overall
evaluation					acceptability
AC (30:70)	8.2	8.4	7.5	7.5	7.6
AC (40:60)	7.5	7.8	7.6	9	6.9
AC (50:50)	7	8.3	7.2	8.6	7.9

TABLE 4

*AC-AMARANTH COOKIES

AMARANTH COOKIES

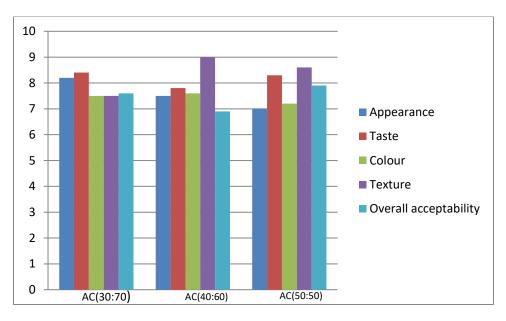


FIGURE-2

The organoleptic evaluations by selected panel members are depicted in table-1 along with graphical representation. Among three samples kept for evaluation, Amaranth cookies 50:50 was the most acceptable one. Therefore, this sample ratio of Amaranth cookies was taken for further study.

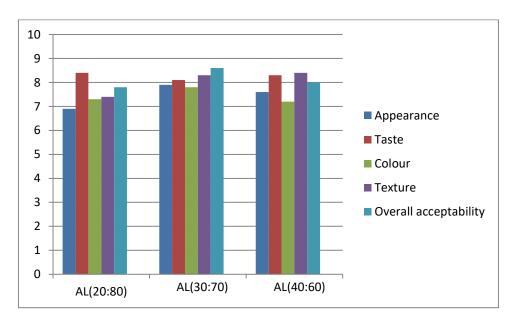
Sensory	appearance	Taste	Colour	Texture	Overall
evaluation					acceptability
AL (20:80)	6.9	8.4	7.3	7.4	7.8
AL (30:70)	7.9	8.1	7.8	8.3	8.6
AL (40:60)	7.6	8.3	7.2	8.4	8.0

4.4.2 AMARANTH LADDOO

TABLE - 5

*AL- AMARANTH LADDOO

AMARANTH LADDOO





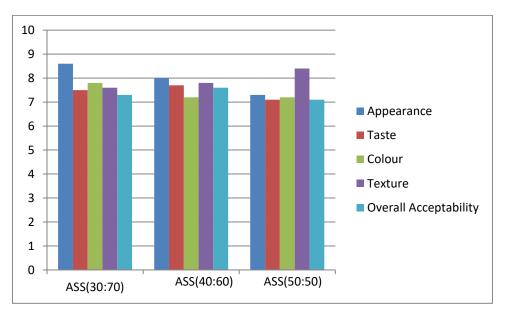
The organoleptic evaluations by selected panel members are depicted in table-2 along with graphical representation. Among three samples variation kept for evaluation, Amaranth laddu30: 70 was the most acceptable among the three different variations for all the ten panel members. Therefore, this sample ratio of Amaranth laddoo was taken for further study.

4.4.3 AMARANTH SPICY SEVAI

Sensory	Appearance	Taste	color	Texture	Overall
evaluation					acceptability
ASS (30:70)	8.6	7.5	7.8	7.6	7.3
ASS (40:60)	8.0	7.7	7.2	7.8	7.6
ASS (50:50)	7.3	7.1	7.2	8.4	7.1

TABLE - 6

*ASS-AMARANTH SPICY SEVAI



AMARANTH SPICY SEVAI

FIGURE-4

The organoleptic evaluations by selected panel members are depicted in table-3 along with graphical representation. Among three samples variation kept for evaluation, Amaranth spicy sevai 40:60 was the most acceptable one. Therefore, this sample ratio of Amaranth spicy sevai was taken for further study.

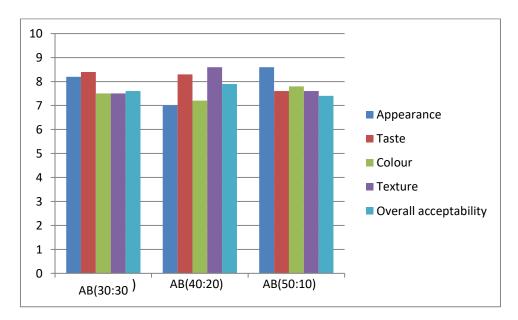
4.4.4 AMARANTH SNACK BAR

Sensory	Appearance	Taste	Colour	Texture	Overall
evaluation					acceptability
AB (30:30)	8.2	8.4	7.5	7.5	7.6
AB (40:20)	7.0	8.3	7.2	8.6	7.9
AB (50:10)	8.6	7.6	7.8	7.6	7.4

TABLE - 7

*AB- AMARANTH BAR







The organoleptic evaluations by selected panel members are depicted in table-4 along with graphical representation. The variation was done changing the quantity of amaranth and oats. Among three samples variation kept for evaluation, Amaranth bar 40:20was the most acceptable one. Therefore, this sample ratio of amaranth bar was taken for further study.

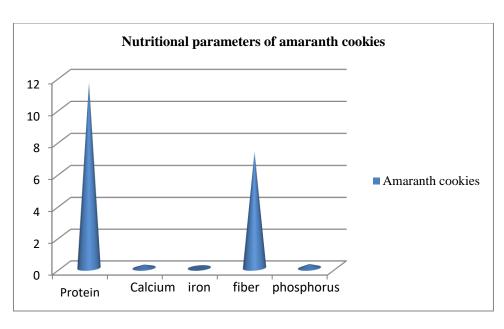
4.5 PHYSIOCHEMICAL ANALYSIS OF SELECTED NUTRIENTS OF DEVELOPED PRODUCT

4.5.1AMARANTH COOKIES

SI.No	NUTRIENTS	AMARANTH COOKIES
		(100g)
1	Protein	11.61g
2	Calcium	0.217 g
3	Iron	0.00592g
4	Dietary fiber	7.28g
5	Phosphorus	0.251g

TABLE -8

The above table represent the total protein, calcium,iron,dietaryfiber, and phosphorus content of amaranth cookies.



FIGURI	E-6
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The nutritional parameters of amaranth cookies were analyzed and elicited in the (Table:5). It contain11.6g protein,0.217g calcium,0.00592g iron,7.28g fiber and 0.251g phosphorus.

4.5.2AMARANTH LADDOO

TABLE -

SI.No	NUTRIENTS	AMARANTH LADDOO (100g)
1	Protein	10.80
2	Calcium	0.111
3	Iron	0.00485
4	Dietary fiber	5.91
5	phosphorus	0. 196

The above table represent the total protein, calcium, iron, dietary fiber, and phosphorus content of amaranth cookies.

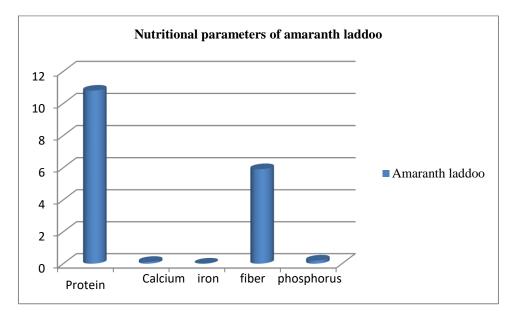


FIGURE-7

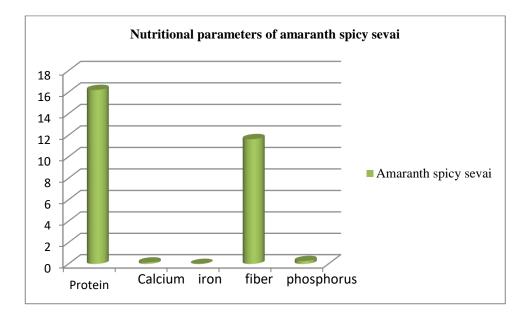
The nutritional parameters of amaranth laddoo was analyzed and elicited in the (Table:6).It contain 10.8g protein,0.111g calcium, 0.00485g iron,5.91g fiber and 0.196g phosphorus.

4.5.3 AMARANTH SPICY SEVAI

SI.No	NUTRIENTS	AMARANTH SPICY SEVAI (100g)
1	Protein	16.21
2	Calcium	0.138
3	Iron	0.00583
4	Dietary fiber	11.64
5	phosphorus	0.277

TABLE –10

The above table represent the total protein, calcium, iron, dietary fiber and phosphorus content of amaranth spicy sevai.





The nutritional parameters of amaranth Spicy sevaiwere analyzed and elicited in the (Table:7). It contains 16.21g protein,0.138g calcium, 0.00583g iron,11.64g fiber and 0.277g phosphorus.

4.5.4 AMARANTH SNACK BAR

SI.No	NUTRIENTS	AMARANTH BAR
		(100g)
1	Protein	9.18
2	Calcium	0.119
3	Iron	0.00387
4	Dietary fiber	8.90
5	phosphorus	0.226

TABLE –12

The above table represent the total protein, calcium, iron, dietary fiber, and phosphorus content of amaranth snack bar.

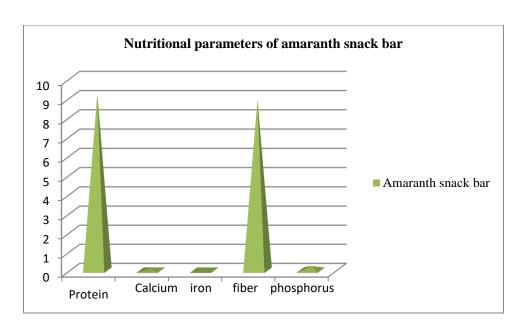


FIGURE-9

The nutritional parameters of amaranth snack bar were analyzed and elicited in the (Table:8). It contains 9.18g protein,0.119g calcium, 0.00387g iron,8.90g fiber and 0.226g phosphorus.

4.6FRIEDMAN'S TEST FOR OVERALL ACCEPTABILITY OF PRODUCTS

4.6.1 AMARANTH COOKIES

Number of panel	Ranks for different variations of cookies		
members(N)	Variation 1	Variation 2	Variation3
1	7	7	9
2	8	8	8
3	7	7	7
4	9	6	9
5	8	8	8
6	7	7	7
7	8	6	6
8	7	7	8
9	7	6	9
10	8	7	8
Total	21	15.5	23.5

TABLE 13

Calculated FM Test Statistics = 3.35

FM Critical value from table (Friedman's ANOVA by ranks critical value table) =6.20

4.6.2 AMARANTH LADDOO

Number of panel	Ranks for different variations of laddu		
members(N)	Variation 1	Variation 2	Variation 3
1	9	9	9
2	7	9	7
3	7	7	7
4	9	9	9
5	8	8	7
6	7	9	9
7	8	9	7
8	6	8	8
9	8	9	9
10	9	9	8
Total	17.5	24.5	18

TABLE: 14

Calculated FM Test Statistics = 3.05

FM Critical value from table (Friedman's ANOVA by ranks critical value table) =6.20

4.6.3AMARANTH SPICY SEVAI

Number of panel	Ranks for different variations of Amaranth spicy sevai		
members(N)	Variation 1	Variation 2	Variation 3
1	6	8	6
2	7	7	7
3	7	8	7
4	7	7	7
5	7	8	8
6	9	7	7
7	7	8	6
8	7	7	7
9	8	8	8
10	8	8	8
Total	19	23	18

TABLE: 15

Calculated FM Test Statistics = 1.4

FM Critical value from table (Friedman's ANOVA by ranks critical value table) =6.20

4.6.4AMARANTH SNACK BAR

Number of panel	Ranks for different variations of Amaranth snack bar		
members(N)	Variation 1	Variation 2	Variation 3
1	7	9	8
2	7	7	7
3	8	8	7
4	7	7	7
5	8	9	7
6	8	8	7
7	7	7	7
8	9	7	9
9	8	9	7
10	7	8	8
Total	19.5	23.5	17

TABLE: 16

Calculated FM Test Statistics = 2.15

FM Critical value from table (Friedman's ANOVA by ranks critical value table) =6.20

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

Amaranthus belonging to the family Amaranthaceae comprises a series of untamed or wild, weedy, and cultivated species and found worldwide in almost all agricultural environments. Amaranthus species have different centers of domestication and origin, being wildly distributed in North America, Central America, and South American Andes where the greatest genetic diversity is found. It is a pseudo grain with high nutritional value as it is rich in protein, essential amino acids, dietary fiber, fat, minerals, vitamins, and natural antioxidants. It also has some some anti-nutritional factors like tannin and phytates in small amount but can be removed easily through washing and proper cooking methods. Amaranth seed has positive effects on metabolic, celiac disease, diabetes, and gastrointestinal health in humans.

Incorporation of amaranth into the diet increases the nutritive value and reduces the risk of various health diseases like cardiovascular diseases, type 2 diabetes, high blood pressure, and celiac disease. Amaranth grain contains large quantities of minerals. The amount of calcium and iron are significantly higher than in the commonly used cereals. Amaranth seed is an option for "gluten-free" meal plans, and it is well tolerated by persons who avoid wheat, including persons diagnosed with celiac disease.

Hence the present study entitled "DEVELOPMENT OF SNACKS BY THE INCORPORATION OF AMARANTH SEEDS" has been undertaken with the following objectives to formulate amaranth snacks, to analyze shelf life of amaranth seed powder, to conduct organoleptic analysis of the developed product, to analyze selected nutrient contents of the developed product.

Being highly nutritious, amaranth can be used to complement the diet of those population who are suffering from malnutrition. Amaranth seed is an excellent source of high nutritionally quality protein whose amino acid composition is rich in lysine and methionine, two limiting amino acids in cereals and legumes respectively and possess high dietary fiber compared to other commonly used cereals.

To use amaranth, grains should be rinsed thoroughly prior to cooking. It is easy to add amaranth to meals as it can be incorporated into diets in a variety of ways- The most frequent mode of consumption of amaranth grains is after popping and milling and by mixing the flour with other cereal flours such as teff, sorghum, barley, and wheat to prepare bread, injera and porridge.

Pale amaranth seed was used to prepare the four snacks, it includes amaranth cookies, amaranth laddoo, amaranth spicy sevai and amaranth snack bar. Three variation of each product were made.

The organoleptic analysis of the samples was done. The sensory evaluation was carried out by 10 untrained panel members. The panel members were provided with samples along with sensory score cards 9-point hedonic scale to rate the products according to their opinion. They were given instructions regarding how to carry out the assessment.

Based on the scores of hedonic rating scale the most accepted among the three variation was identified.

The salient finding was,

- The shelf life of amaranth seed powder was analyzed by comparing fresh sample and the sample after 3 months.
- The fresh samples shelf life analysis shown that it contains 4.99g moisture, 460CFU Aerobic plate count, and< 100CFU yeast and mould rate.
- The sample after 3 month shelf life analysis shown that it contains 13.27g moisture, 24000CFU Aerobic plate count and 400CFU yeast and mould.
- Among three samples variation kept for evaluation, 50:50 was the most acceptable of Amaranth cookies were taken for evaluating nutritional content and packaging and labeling.
- Among three samples variation kept for evaluation, 30: 70 was the most acceptable Amaranth laddoo sample and that was taken for evaluating nutritional content and packaging and labelling
- Among three samples variation kept for evaluation, 40:60 was the most acceptable Amaranth Spicy Sevai sample. The selected variant was taken for evaluating nutritional content and packaging and labeling
- Among three samples variation kept for evaluation, 40:20was the most Acceptable. Amaranth snack bar sample and that was taken for evaluating nutritional content and packaging and labeling.
- The nutritional content of amaranth cookies was analyzed, and it has 11.61g protein, 216.71mg calcium ,5.92 mg iron, 7.28 g dietary fiber and 250.68 mg phosphorus.

- The nutritional content of amaranth laddoo was analyzed and it has 10.80 g protein, 111.17mg calcium ,4.85 mg iron, 5.91 g dietary fiber and 196 mg Phosphorus.
- The nutritional content of amaranth spicy sevai was analyzed and it has 16.21 g protein, 138.20mg calcium ,5.83 mg iron, 11.64g dietary fiber and 276.74 mg phosphorus
- The nutritional content of amaranth snack bar was analyzed, and it has 9.18g protein, 118.70mg calcium, 3.87 mg iron, 8.90g dietary fiber and225.90 mg phosphorus.
- Friedman's test for amaranth cookies showed that the overall acceptability of all the samples were different
- Friedman's test for amaranth laddoo showed that the overall acceptability of all the samples were different.
- Friedman's test for amaranth spicy seval showed that the overall acceptability of all the samples were different.
- Friedman's test for amaranth snack bar showed that the overall acceptability of all the samples were different.

Conclusion

In this study, Amaranth seed was incorporated with variety of sweet snacks. It can be incorporated with any food of our choice and was acceptable for everyone. The incorporation of amaranth seeds does not bring any taste variations in food. The development of all food products was carried out under hygienic condition with little or no wastage of food materials. The food products were prepared without addition of preservatives.

Because of high protein content in amaranth snacks, it benefits in combating malnourishment in children . Also Amaranth seed are safe and beneficial for lactose intolerant kids and celiac patients by eliminating the triggering factors like milk and wheat in products.

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ESTIMATION OF PROTEIN

Objective:

To determine the total protein present in developed products

Application and limitation:

Applicable to food items. Total protein by Kjeldhal method is defined as the amount of nitrogen experimentally found and multiplied by an appropriate conversion factor.

Principle:

The sample is oxidized by the presence of sulphuric acid and nitrogenous compounds are converted into Ammonium sulphate. Mercury is added to the digestion mixture as a catalystand alkaline sulphate as a boiling elevator. Ammonia is liberated by adding an excess of alkaliand is quantitatively distilled into a measured volume of standard alkali.

Apparatus and Reagents:

Kjeldhal Digestion and Distillation assembly, Balance readable to 0.1 mg, Burette beakers, Sodium hydroxide solution=0.1N-Kjeldhasl nitrogen, Concentrated sulphuric acid 93-98% by mass -Nitrogen free, Mercuric oxide or metallic mercury-Nitrogen free, Sodium sulphat eo ranhydrous potassium sulphate, Zinc granules, Sulphuric acid 0.1N

standard solution (5gmH2SO4 make up to 12 of D/W), Methyl red indicator, Dissolve 250 mg Methyl red in 100ml.Ethanol, Strong solution of Sodium hydroxide (50%),4% Potassium or sodium sulphide

Procedure:

- Weigh accurately 0.7 -2.2 gm of test sample into the Kjeldhal flask, taking precautions to see that particles of the material do not stick on to the neck of the flask
- Add 0.7gm HgO or 0.65gm metallic Hg
- Add 15g powdered K₂SO₄/anhydrous Na₂SO₄
- Add 25 ml conc.H₂SO4, If test portion >2.2gm is used .Increase H₂SO₄ by 10 ml for each gm test portions .
- Place flask in inclined position and heat gently until frothing ceases
- Increase the temperature ,digest for more than 2 hours until the solution clears
- Cool the contents of the flask –Transfer it quantitatively to the KB flask with Ca 200ml H₂SO₄, add 25ml of potassium or sodium sulphide solution and mix to precipitated Hg.
- Add few Zn granules to prevent bumping
- Add layer of Na OH solution without agitation .Immediately connect this Kjeldhal flask with the protein distillation apparatus, with tip of condenser immersed in standard H₂SO₄ and 5-7 drops indicator in receiver
- Mix the contents of the flask by shaking and distill until all the ammonia has passed over in the standard H₂SO₄

- Shut off the burner, remove receiver and rinse the condenser thoroughly with water into the receiver. Wash the tip of the condenser into the receiver.
- Titrate excess standard acid in distillate with standard NaOH solution
- Correct for blank determination on reagents

Calculations:

Nitrogen=(B-S) ×N×0.014×100/W

Were,

B=ml of NaOH used for blank titration

S=ml of NaOH used for sample titration

N=Normality of NaOH

W=Weight of sample in gm

Protein =Nitrogen % × Protein factor

DETERMINATION OF CALCIUM

Objective:

To determine the calcium present in the developed products

Applications and limitations:

This method is used to find the calcium content of milk, the hardness of water and the amount of calcium and calcium carbonate in various solid materials. It is a very established, reliable and accurate method but it takes time to complete as done manually.

Reagents:

NaOH-1 N, Murexide solution (ammonium purpurate) -0.100mg of murexide with 10g of solid sodium chloride and grinding the mixture to 300-425 microns, Standard EDTA solution =0.01N

Procedure:

- Weigh accurately 5-10g of the material in previously weighed crucible.
- Dry for 2 hours in an air oven maintained at 105±2°C and ignite the divided material in the dish with the flame of a burner for about 1 hour.
- Complete the ignition by keeping in a muffle furnace at 600±20°C until grey ash results.
- Cool in a desiccator and weigh

- Repeat the process of heating for 30 minutes, cooling and weighing till the difference in mass between two successive weighing is less than 1mg.
- This ash is then dissolved in minimum amount of concentrated HCL and made upto 100ml using distilled water.
- Take 50 ml of thissample.Add 1 N NaOH solution, a volume sufficient to produce a PH of 12-13.
- Add 2-3 drops of the murexide indicator.
- Titrate against EDTA with continues stirring.
- The end point is indicated by color change from pink to purple.

Calculation

Calcium= (Titrate value*normality of EDTA*40.08*1000*dilution)/volume taken for test

DETERMINATION OF IRON

Objective:

To determine the Iron present in the developed products

Applications and limitations:

This method is applicable to determine Iron by dry ashing and Flame Atomic Absorption Spectrometry

Apparatus and reagents:

Air oven, Muffle furnace, Desiccator, Spectrophotometer, ConcentratedHCL, Hydroxylamine hydrochloride, Ammonium acetate buffer, 1,10Phenanthrolin solution

Procedure:

- Weigh accurately 5-10g of the material in previously weighed crucible.
- Dry for 2 hours in an air oven maintained at 105±2°C and ignite the divided material in the dish with the flame of a burner for about 1 hour.
- Complete the ignition by keeping in a muffle furnace at 600±20°C until grey ash results.
- Cool in a desiccator and weigh
- Repeat the process of heating for 30 minutes, cooling and weighing till the difference in mass between two successive weighing is less than 1mg.

- This ash is then dissolved in minimum amount of concentrated HCL and make up to 100ml using distilled water.
- To 100ml of this sample and 50ml of blank, add 1ml hydroxylamine hydrochloride and add 0.5ml con. HCL.
- Then boil the contents to almost 20ml the volume for dissolution of all ions.
- Cool to room temperature. Add 10ml of ammonium acetate buffer and 10ml of 1,10Phenanthrolin solution and mix well.
- Dilute to 100ml and place each standard solution and food solution into a separate cuvette.
- Measure and record the absorbance of each solution at 510nm using spectrophotometer.
- Prepare a standard curve (Beer's Law) of the standard concentrations vs. absorbance.

Calculation:

The amount of Iron present in the sample can be calculated from the standard graph in mg/dl.

DETERMINATION OF PHOSPHORUS

Objective:

To determine the phosphorus present in the developed products

Application

These methods cover the determination of specified forms of phosphorus in drinking, surface and saline waters, domestic and industrial wastes. The methods are based on reactions that are specific for the orthophosphate ion. Thus, depending on the prescribed pretreatment of the sample, the various forms may be determined.

Apparatus

Spectrophotometer, Crucibles, Analytical balance, Volumetric flasks, Muffle furnace, Filter paper., Hotplate, Water bath, Metalbasket, Weights, Cuvettes

Reagents

Hydrochloric acid, Zinc oxide., Potassium hydroxide solution, Sulfuric acid, Sodium molybdate solution, Ascorbic acid solution, Molybdate-ascorbic acid solution, Phosphorus stock standard solution, Phosphorus working standard solution, Phosphorus solutions for standard curve.

Preparation of Test Sample

- Accurately weigh 0.5-1.5 g (± 1 mg) homogeneous test sample into crucible. To control possible contamination, prepare reagent blank by including an empty crucible in analytical run. Treat reagent blank in the same manner as test sample.
- Add 0.5 g zinc oxide into test sample and mix with glass rod; leave glass rod in crucible. Dry sample 1-2 h at ca 110°C
- Pre-ash sample on hot plate until residue is black. {Note: No pre-ashing is needed if furnace used in next step is equipped with a time-temperature regulator.) Place crucible in muffle furnace at room temperature, and let temperature rise to 525°C. Maintain this temperature 4 h or overnight. When using furnace equipped with a time-temperature regulator, use slow initial increase of temperature to avoid the risk of splashing liquid samples.
- Remove crucible from oven and let cool to room temperature. To cold crucible, add 5 mL H20 and 5 mL HC1. Cover crucible with watch glass and boil its contents carefully 5 min on hot plate.
- Filter contents of crucible into 100 mL volumetric flask. Rinse inner surface of watch glass with 5 mL hot H20. Repeat rinsing 4 times with 5 mL hot H20 and collect all rinses in volumetric flask.
- Cool flask to room temperature and neutralize solution by adding 50% KOH solution until solution is slightly opalescent [Zn (OH)2]. Add HC1 dropwise until opalescence disappears. Add 2 extra drops of HC1. Let solution cool to room temperature and then dilute to 100 mL with H20.

- Depending on the expected content of phosphorus, accurately pipet 1.00-10.0 mL treated solution into 50 mL volumetric flask. Dilute solution to 15 mL with H20.
- Add 20 mL molybdate-ascorbic acid solution to test solution in 50 mL flask, and also to phosphorus standard solutions, C(j)- Swirl contents carefully.
- Place flasks in metal basket. Close each flask with stopper, inserting narrow filter paper strip at the stopper so that flask is not closed too tightly.
- Place lead wire or stainless-steel nut on flask as a weight. Immerse metal basket in vigorously boiling water bath. Keep flasks in water bath exactly 15 min. Cool flasks under tap H20 to 20°-30°C, and then dilute contents to 50 mL with deionized H20 and mix.

Calculations

Calculate phosphorus content as phosphorus in test sample (g/100 g) as follows: Phosphorus, g/100 g = 100 x [(V2/V {) x P]/W

DETERMINATION OF TOTAL DIETARY FIBER

Objective:

To determine the Dietary fiber present in the developed products

Application:

This method determines soluble, insoluble, and total dietary fiber content in processed foods and raw materials, such as cereal products, fruits and vegetables.

Apparatus:

Dispenser, Fritted crucible, Gooch, fritted disk, Pyrex® 50 mL. Filtering flask, Rubber ring adaptors for use on filtering flasks, Vacuum source, Water bath, Balance, Ovens, Timer, Desiccator, pH meter, Dispensers, Cylinder, Magnetic stirrers and stirring bars, 16. Rubber, spatulas, Muffle furnace

Reagents:

Phosphate buffer, 0.08 M, pH 6.0. Dissolve 1.400 g disodium phosphate anhydrate (Na2HPO4) (or 1.753 g dihydrate) and 9.68 g disodium phosphate monobasic monohydrate (NaH2PO4) (or 10.94 g dihydrate) in approx. 700 mL distilled water. Dilute to 1 L with water. Check pH with pH meter.

- Sodium hydroxide solution, 0.275 N. Dissolve 11.00 g ACS grade NaOH in approx. 700 mL distilled water, using appropriate handling precautions, in 1 L volumetric flask. Cool and dilute to volume with water.
- iii. Hydrochloric acid solution, 0.325 N. Dilute stock solution of known titer (i.e., 325 mL of 1.0 N HCl) to 1 L with water in volumetric flask.

Procedure

- Weigh duplicate 1 g samples, accurate to 0.1 mg, into 400 mL tall-form beakers. Sample weights should differ by less than 20 mg from each other. Add 50 mL phosphate buffer (pH 6.0) to each beaker and check pH with pH meter. Adjust if pH does not equal 6.0±0.1.
- Add 50 μ L heat-stable α -amylase solution.
- Cover beaker with aluminum foil and place in boiling water bath. Beaker must be incubated at 98-100°C for 15 min. Shake gently at 5 min intervals. Note: Increase incubation time when number of beakers in bath makes it difficult for beaker contents to reach internal temperature of 98-100°C. Use thermometer to indicate that 15 min at 98-100°C is attained. Total of 30 min in boiling water bath should be sufficient.
- Cool solutions to room temperature.
- Adjust to pH 7.5±0.1 by adding 10 mL 0.275 N NaOH solution. Check pH with pH meter.
- Add 100 µL of protease solution.
- Cover beaker with aluminum foil and incubate at 60°C with continuous agitation for 30 min.

- Cool and add 10 mL 0.325 N HCl solution to adjust pH to 4.5±0.2. Check pH with pH meter.
- Add 200 μL amyl glucosidase, cover with aluminum foil, and incubate for 30 min at 60°C with continuous agitation.
- Add 280 mL 95% EtOH pre-heated to 60°C (measure volume before heating).
 Let precipitate form at room temperature for 60 min.
- Weigh crucible containing Celite to nearest 0.1 mg, then wet and distribute bed of Celite in crucible by using stream of 78% EtOH from wash bottle.
- Apply suction to draw Celite onto fritted glass as even mat. Maintain suction and quantitatively transfer precipitate from enzyme digest to crucible.
- Wash residue successively with three 20 mL portions of 78% EtOH, two 10 mL portions of 95% EtOH, and two 10 mL portions of acetone. In some cases, gums may form during filtration, trapping liquid in residue. If so, break surface film with spatula to improve filtration. Long filtration times can be avoided by careful intermittent suction throughout filtration.
- Dry crucible containing residue overnight in 70°C vacuum oven or 105°C air oven.
- Cool in desiccator and weigh to nearest 0.1 mg. Subtract crucible and Celite weights to determine weight of residue.
- Analyse residue from one sample of set of duplicates for protein by using N x
 6.25 as conversion factor.

 Incinerate second residue sample of duplicate for 5 h at 525°C. Cool in desiccator and weigh to 0.1 mg. Subtract crucible and Celite weights to determine ash.

Dietary Fiber (%) =
$$\frac{\frac{R_1 + R_2}{2} - p - A - B}{\frac{m_1 + m_2}{2}} \times 100$$

Where,

R1 = residue weight 1 from m1; R2 = residue weight 2 from m2 m1 = sample weight 1; m2 = sample weight 2 A = ash weight from R1; p = protein weight from R2 and

$$B = blank = \frac{BR_1 + BR_2}{2} - BP - BA$$

Where

BR = blank residue.

BP = blank protein from BR1 BA = blank ash from BR2.

SENSORY EVALUATION CARD

NAME

DATE

INSTRUCTIONS TO FOLLOW

:

:

Please rinse your mouth with water before starting. You may rinse your mouth again at any time during testing if you need to. Please taste the three sample in order presented from left to right. You may re taste the samples, once you have tried all of them score the samples within the possible scores.

9 hedonic score rating card:

	AMA	RAN	TH	AMAR	ANTH		AMA	RAN	TH	AMA	RAN	ΓН
Sensory characteristics	COOKIES			LADDOO			SPICY SEVAI		SNACK BAR			
	А	В	С	А	В	С	А	В	C	А	В	C
Appearance												
Colour												
Taste												
Texture												
Overall acceptability												

Please evaluate the sample by ticking on any of the number according to your perception

9 = Like extremely	4 = Dislike slightly
8 = Like very much	3 = Dislike moderately
7 = Like moderately	2 = Dislike very much
6 = Like slightly	1 = Dislike extremely

5 = Neither like nor dislike

THANK YOU FOR PARTICIPATION

FRIEDMAN'S ANOVA BY RANK CRITICAL VALUE TABLE

k=3			
Ν	α <.10	$\alpha \leq .05$	α <.01
3	6.00	6.00	
4	6.00	6.50	8.00
5	5.20	6.40	8.40
6	5.33	7.00	9.00
7	5.43	7.14	8.86
8	5.25	6.25	9.00
9	5.56	6.22	8.67
10	5.00	6.20	9.60
11	4.91	6.54	8.91
12	5.17	6.17	8.67
13	4.77	6.00	9.39
∞	4.61	5.99	9.21

k=4

N	α <.10	$\alpha \leq .05$	α <.01
2	6.00	6.00	
3	6.60	7.40	8.60
4	6.30	7.80	9.60
5	6.36	7.80	9.96
6	6.40	7.60	10.00
7	6.26	7.80	10.37
8	6.30	7.50	10.35
∞	6.25	7.82	11.34
k=4			
Ν	α <.10	$\alpha \leq .05$	α <.01
3	7.47	8.53	10.13
4	7.60	8.80	11.00
5	7.68	8.96	11.52
∞	7.78	9.49	13.28