Development And Quality Control Analysis Of Herbal Bakery Nutraceutical Product

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Abstract - A nutraceutical product herbal bread has been developed through our research work for development of color, flavour, texture, taste, overall acceptability and shelf life of traditional bread sample. With the herbal bread, different types of analysis like Sensory Analysis and ANOVA has been carried out to optimise the sample. The mostly accepted samples in our study consist of {herbs(5% & 15%w/w), spices(8%w/w), phycoerythrin(20%v/w), ascorbic acid(10%v/w), yeast broth(5%w/w) and sugar(5%w/w)}. These bread samples kept at ordinary and ambient temperatures and refrigerated temperatures showed higher shelf life than ordinary one.

Index Terms - phycoerythrin, Amaranthus cruentus, yeast, aerobic, fermentation, putrefaction, dough, spices, baking, maillard browning, sensory analysis, ANOVA.

I. INTRODUCTION

Bakery items has been the most oldest and ardent staple products till date which was centralized around 15th century even prior to 18th century industrial revolution. The explications of cultivating yeasts added feather to the art of baking with fine grinds of various cereals and grains that has been discovered on course of time. The development of Sourdough and its applicability with different trends of food making pertaining from continental to tropical and culinary to cuisines have been a major turn over in the kitchen artistry. Of the several bakery products consumed, bread has been the most popular. The protocols for baking achieved numerous variations in order to have a more tastier, smoother, healthier, attractive levels with equivalent nutritive and dietetic values. Among these Amaranth the peculiar pseudocereal has found its propound interests in widespread bakery deployments. Studies revealed that amaranth flour is a good source of proximate principles of food and also medicinal therapeutic aspects to serve best for clinical ailments, disparities, diseases and biochemical as well as cellular anomalies due to the presence of high quantities of minerals, alkaloids, secondary metabolites and vitamins which contributed to develop high quality gluten matrix free dough with crude (proteins, fats and fibres) with better physical appearance of color, aroma, flavour, better textural qualities of less (hardness and bake loss), increased (springiness and cohesiveness) hence better sensory and agronomic importance over traditional wheat flour.(0) In fact the breads enriched with Amaranth extracts were preferred the most than with other herbs in terms of appearance, acceptability and sensory attributes.(0) Increased effectiveness of amaranth extract mixed with all purpose and whole wheat flour has resulted in enhanced properties of water holding capacity, dough development timing, softness and elasticity along with food grade protein and enriched nutritive values than the traditional breads.(0) Besides the addition of Amaranthus grain flour cultivars yielded better quality of composite bread with improved medicinal aspects such as balancing vascular, coronary, cardiac, hormonal, neuronal and somatic anomalies due to upgraded nutritive and sensory proportions compared to traditional breads.(0)

II. GLOBAL SCENARIO

2.0 Literature Survey

Survey of literature of a particular subject is helpful in understanding the conceptual framework and provides a detailed account of work, which has been done in the past on that particular subject. It supports the candidate in deciding the line of action to complete the research work.

Here are some of the excerpts of some of the research works which were done in the past by notable research scholars:

A quintessential study was done at different levels of amaranth grain flour0 to 50% (w=w) mixed with the wheat flour and other ingredients (1% salt, 2.5% fat, 1.5% yeast, 10% sugar and 52774% water), fermented, molded, pan-proved and baked. The baked products were evaluated for loaf volume, moisture content and sensory qualities (color, odor, taste and texture) and compared with bread made from 100% wheat flour. The water absorption of the composite flour increase with increased in level of amaranth grain flour. The loaf volume index decreased from 3.29 to 1.9 and the moisture content increased from 22 to 42% with increase in amaranth grain flour. The sensory means scores of the odor taste, colour and texture decreased from 6.9 to 4.0, 7.1 to 4.8, 7.1 to 6.8 and 6.9 to 4.7 respectively. Generally, above 15% (w=w) amaranth grain flour, there were significant different ($p \leq 0.05$) in the evaluated sensory qualities and the product unacceptable.

Ayo J. A. (2001).(0)

To investigate the volatile fractions of 16 essential oils for activity against the more common fungi causing spoilage of bakery products, *Eurotium amstelodami, E. herbariorum, E. repens, E. rubrum, Aspergillus flavus, A. niger and Penicillium corylophilum* strains were examined.
The study applied 50 µl of pure essential oils in a sterilized filter paper, was carried out at pH 6 and at different water activity levels (0.80–0.90). First, a wheat flour based agar medium was used, where cinnamon leaf, clove, bay, lemongrass and thyme essential oils where found to totally inhibit all microorganisms tested. These five essential oils were then tested in sponge cake analogues, but the antifungal activity detected was much more limited.
Five essential oils showed potential antifungal capacity against all species tested, over a wide range of water availability. Their activity, however, seems to be substrate-dependent. More research is needed to make them
work in real bakery products, as in the preliminary study limited effectiveness was found. The potential of the cinnamon leaf, clove, bay, lemongrass and thyme essential oils against species belonging to *Eurotium, Aspergillus and Penicillium* genus has been demonstrated.
Guynot M. E., Ramos A. J. *et al.* (2003 Jan 31).(0)

A look over had also been made on the effect of ascorbic acid on the rheological properties of wheat fermented dough from forty three wheat flour samples, represented two groups of flours, characterised (according to the ash content, protein content and Zeleny sedimentation value) the main Czech flour type has been studied. Standard analytical parameters (ash and protein contents, wet gluten, falling number, Zeleny sedimentation value), rheological investigation (maturograph, oven rise recorder), and laboratory baking test were used for the characterisation of flours and doughs. It was stated that the influence of the ascorbic acid addition on the fermented dough behaviour depends on the flour composition particularly in the proofing stage. Oven rise characteristics of dough and specific bread volume revealed smaller changes without significant differences between flours with lower (up to 0.6%) and higher (up to 0.7%) ash contents. An important correlation (r = 0.51–0.68) significant at 0.01 level has been found between specific bread volume and final rise of dough.
HRUŠKOVÁ MARIE and NOVOTNÁ DANA (2003).(0)

Again analytical Investigations on the impact of commercial products with complex additive (0.1, 0.3, and 0.5%), L-ascorbic acid (0.002, 0.004 and 0.012%), diacetyl ester of tartaric acid with monoglycerides (DATEM E472e, 0.1, 0.3, and 0.5%), α-amylase (0.002, 0.006 and 0.012%), xylanase (0.004, 0.012 and 0.024%), alcohol extract of rosemary, thyme or sage (0.5, 1.0 and 2.0%), as well as the combination of complex additive and rosemary, thyme and sage extract on rheological characteristics of dough was routined. The study included amylograph, farinograph and extensograph analysis of dough with and without additives (control sample). The volume of lost CO2 gas (mL) was the lowest in dough samples with an added combination of complex additive and thyme extract (0.05 and 0.5%) and rosemary extract (2.0%). In the samples with thyme extract (1.0%) added, the volume of lost gas was at a level of samples with added complex additive, DATEM, and L-ascorbic acid.
DAVIDOVIĆ DEJAN N., DODIĆ SINIŠA N. *et al*. (2010 Jul 16).(0)

On the other hand the effects of amaranth and quinoa flours and protein isolates prepared from amaranth and quinoa seeds on the rheological properties of wheat flour dough an bread were studied using new recording instruments, the micro Z-arm mixer (for dough) and the SMS-Texture analyser (for bread crumb). The addition of 10% amaranth or quinoa flours did not cause significant changes in rheological properties. However, higher additions (20% and 30%) resulted in significant changes in stability, the degree of softening and elasticity. Substitution of wheat flour by amaranth or quinoa flours resulted in an increase of water absorption capacity. A significant reduction of specific volume and an increase of resistance to deformation (firmness) of the crumb of breads prepared from flour mixtures containing high percentages of amaranth or quinoa flours was observed. The addition of protein isolates did not significantly influence the main rheological parameters of dough, and bread crumb.
Tömösközi Sándor, Gyenge Lilla et al. (2011).(0)

Besides, the use of composite coriander-wheat flour for commercial bread making purposes and consumption of coriander leaf fortified bread were relatively new. Response surface methodology was used to study the effects of baking conditions on various baking parameters and thus the optimum conditions selected for further studies. Time and temperature were the two important baking conditions on which the quality and acceptability of the breads depend. In this study, baking temperature and time were the predictor variables and loaf specific volume, crumb moisture and crumb hardness were the dependent variables (responses). Baking temperature and time ranged from 200 to 240ºC and 10 to 30 min respectively. Loaf weight, volume, and the color of the crumb and crust of the bread samples varied significantly with the baking time and temperature. Quadratic model fitted with the experimental data of specific volume, crumb moisture and hardness obtained. The baking conditions of the herbal bread had been optimized with the help of RSM.
Das Lipi, Raychaudhuri Utpal *et al.* (2012 May).(0)

On the other hand it was reported that the polyols namely glycerol, sorbitol and mannitol incorporated at 2, 4 and 6%level in flour for bread making and their effect on textural properties, bread making quality and sensory acceptability of bread was studied. The effect of incorporation revealed the increased bake absorption, bread weight and decreased specific volume. The overall acceptability scores were maximum for bread prepared with glycerol at 2 % level, followed by sorbitol at 4 % level and mannitol at 4 % level. During storage of packed bread, moisture content and water activity were higher for bread prepared from polyols as compared to control and it was observed that moisture content was higher in bread packed in Polypropylene. Formation of free fatty acid content (% oleic acid) was observed to be higher in the breads stored at ambient condition and

packed in Low density polyethylene packaging material. The overall acceptability of bread decreased with the increased storage period.
Bhise Suresh & Kaur A. (2014 Jan 22).(0)

The next study was aimed at improving bread-making performance of two spelt cultivars from organic production differing in dough rheological properties. For this purpose, a small composite design was employed to study the combined effect of xylanase (XYL), ascorbic (ASC) and citric (CIT) acid on bread characteristics and, finally, to optimize their doses. In both cultivars, XYL exerted improving effects on specific volume and crumb firmness. In stronger spelt cultivar, there was a significant ASC/XYL synergistic effect on improving loaf volume and crumb properties. CIT showed crumb softening effect in both cultivars and increased crumb resilience (weaker spelt being more sensitive). Optimized formulation contained (in mg/kg): 50 ASC/90 CIT/60 XYL for stronger and 20 ASC/10 CIT/120 XYL for weaker spelt cultivar. The optimized formulations allowed substantial increases in specific volume (by 45%) and crumb resilience (30%) for stronger and crumb softening (36%) for weaker spelt cultivar.
FILIPCˇ EV BOJANA, ŠIMURINA OLIVERA *et al.* (2014 Jan 13).(0)

In the next study 12 % mixture of mashed carrot and pumpkin and powder of pumpkin seeds and medicinal herbs were utilized. St.-John's-wort *(Hypericum L*); thousand-leaf (*Achilea millefolium L.*) and licorice root (xty.beat) were used to enrich dietetic bakery product - tapa-nan produced with first grade wheat flour. The effect of addition on dough on its rheological properties, on bread on its physical-chemical and sensory parameters of bread was studied. The results showed that the baking properties of flour and the rheological properties of the dough with additives at a dosage 12% to weight fraction of flour increased significantly ($p<0.05$). It was found that the introduction of mixture has intensifying effect on the fermentation process, reduces gluten content and strengthens its structural and mechanical properties. Also the reduction of the dough fermentation period and expenses of dried substances were found. Introduction of functional additives increased the water absorption capacity of flour (WAC), lowered dilution of dough consistency; as well its elasticity improved.
Physical – chemical parameters of the proposed dietetic national bakery product (tapa-nan) such as moisture, porosity, titratable acidity showed no significantly ($p<0.05$) different. In appearances, taste, smell and flavor definitions showed that addition of 12 % mixture of mashed carrot and pumpkin and powder of pumpkin seeds and medicinal herbs: St.-John's-wort (*Hypericum L*); thousand-leaf (*Achilea millefolium L.*) and licorice root (xty.beat) had more to yellowness. In texture characteristics, the result showed the hardness of bread decreased and chew ability improved. Apart from that, biological value of enriched bread increased.
Beisenbayev Anvarbek Yusupovich, Myrkhalykov Zhumakhan Ushkempirovich *et al.* (2015 Aug 30).(0)

Bread was also produced from wheat flour and fermented unripe banana using the straight dough method. Matured unripe banana was peeled, sliced, steam blanched, dried and milled, and sieved to obtain flour. The flour was mixed with water and made into slurry and allowed to stand for 24h after which it was divided into several portions and blended with wheat flour in different ratios. Proximate and mineral compositions as well as functional, pasting, and sensory characteristics of the samples were determined.
Addition of unripe banana was found to increase the crude fiber, ash, iron, and zinc content of the bread samples. Meanwhile, the products were acceptable by the sensory panelists although a significant difference was observed between the control and the experimental samples used in these studies. There was no conflict of interest.
Adebayo-Oyetoro Abiodun Omowonuola, Ogundipe Oladeinde Olatunde *et al.* (2015 Aug 27).(0)

A study examined the effects of whole amaranth substitutions at various proportions and evaluated the cookies baking behavior. Six types of formulations of cookies were prepared with whole amaranth flour ranging from 20, 40, 60, 80, and 100%. These cookies were evaluated for physical (thickness, diameter, spread ratio, and bake loss), textural, and organoleptic attributes. The diameter and spread ratios were found to be higher in whole amaranth flour cookies 52.20 mm and 6.46, respectively, as compared to other blends (20–80%) of cookies from 51.37 to 51.92 mm and 6.13 to 6.36, respectively. Textural measurement showed that hardness of cookies decreased with the addition of amaranth flour. Whole amaranth flour cookies required least snap force (72.4 N) compared to control (wholewheat flour) cookies (145 N). Sensory data indicated that the amaranth cookies with up to 60% were acceptable, while additional amaranth flour resulted in a decreased mean score for overall acceptability.
Chauhan Arti, Saxena D.C. et al. (2016 Jan 08).(0)

Another study investigated the effect of supplementation of the leaf powders of Telfairia occidentalis, Amaranthus viridis, and Solanum macrocarpon on the chemical composition and the quality characteristics of wheat bread. The bread samples were supplemented with each of the vegetable leaf powders at 1%, 2%, and 3% during preparation. The bread samples were assayed for proximate composition, mineral composition, physical, sensory, and antioxidant properties using standard methods.Theaddition of vegetable powders significantly increased the protein (9.50 to 13.93%), fibre (1.81 to 4.00%), ash (1.05 to 2.38%), and fat (1.27 to 2.00%). Supplementation with vegetable powder however significantly decreased ($p < 0.05$) the carbohydrate and moisture contents. Significant ($p < 0.05$) increaseswere recorded for all evaluatedminerals as the level of vegetable powder increased. Supplementation with vegetable powder caused significant decrease in total phenolic content, percentage DPPH inhibition, metal chelating ability, ferric reducing antioxidant power, and total antioxidant capacity. Sensory results showed that there was significant decrease in sensory qualities with increasing supplementation. This therefore suggests that bread supplemented with vegetable powder could have more market penetration if awareness is highly created. Odunlade T. V., Famuwagun A. A. et al. (2017 Oct 30).(0)

2.1 Hypothesis of Research Study

Based on previous research works our present study is aimed at the following presumptions:

1) Enhancement of Sensory Property of traditional bread by using Herbs and Spices.
2) To develop a nutraceutical product as Herbal Bread.
3) To enhance shelf life of the said product.
4) To enhance the flexibility, consistency and market assurance of our developed product with high esteem of qualitative inclinations.
5)

III. MATERIALS

3.0 Organic ingredients

1) Amaranth (*Amaranthus cruentus*) (200 gm)
2) Herbal Products:-(Clove, Cardamom, Nutmeg, Cumin, Fenugreek, Cinnamon, Mustard, Aniseed, Mace & Black Pepper) (50 gm each packet)
3) Grapes (350 gm)
4) Flour (5 kg)
5) 2 Lemons

3.1 Instruments

1) Plastic jar (2 Litter)
2) Small 200 ml plastic containers(10)
3) Stainless steel forceps, scissors, stirrer, pots
4) 120 ml bone china cup
5) Measuring cylinder (250 ml)
6) 18 Test tubes (15x150mm)
7) Non-Absorbent cotton(25 gm, 1 packet)
8) Cotton cloth(1'x1')
9) 2.5 ml glass dropper
10) pH paper (1 container)

3.2 Reagents, media and chemicals

1) Ethanol (Absolute 99.9%)
2) De-ionized/Distilled water

IV. METHODOLOGY

4.0 Pretreatment of Herbs

a) At first the plant shoot is washed in running tap water nicely to remove all the dirts, soil, pollens and worms or insects if present.
b) The plant shoot is then soaked in distilled water **for 15-20 minutes** by shaking it continuously.
c) Now the shoot is soaked into **2% and 5% sodium chloride (NaCl) solution for about 15-20 minutes and again 15 minutes consecutively** with continuous agitation for primary sterilization to remove bacterial, viral, protozoan, parasitic cysts, colonies, species, infestations and for secondary sterilization to remove fungal, bacterial and other microbial endotoxins and harmful end products respectively.
d) Now the shoot is washed in **Distilled water(D/W) 3-4 times** to remove traces of NaCl.
e) Now the plant is completely sterilized and free off any contaminant and is subjected to further experimental purposes.

4.1 Blanching of Herbs

The de-pigmentation of **The Common Indian Amaranth (*Amaranthus cruentus*)** is done in three statistics. The fresh deep red/pink/reddish green coloured leaves only are selected and cut from **apical, intercalary, axillary, lateral nodes** of the entire shoot with a disinfected highly sterilized stainless steel scissor. It is made sure that while cutting the leaves the cuts are precisely made from leaf stalk keeping the entire leaf lamina undisturbed from any damage. Then those fresh leaves are inserted into **9 test containers of 80x60mm(200ml)** size according to the following protocols. Insertion is done by highly sterilized disinfected stainless steel forceps followed by non-absorbent cotton plugging necessarily.

Fig.01

The blanching processes observed in Organic Solvent like Ethanol(CH3CH2OH) then in Luke warm distilled water and lastly in 50% ethanol+50% Luke warm D/W are given in following data tables:-

Table 4.1: (Test for CH3CH2OH)

Test tube No.	Wt. of leaves	Vol. of CH3CH2OH
01	5gm	200ml
02	10gm	200ml
03	15gm	200ml

Table 4.1: (Test for Luke warm D/W)

Test tube No.	Wt. of leaves	Vol. Of Luke warm D/W
01	5gm	200ml
02	10gm	200ml
03	15gm	200ml

Table 4.1: (Test for 50%CH3CH2OH + 50%Luke warm D/W)

Test tube No.	Wt. of leaves	Vol. Of CH3CH2OH +Luke Warm D/W
01	5gm	200ml
02	10gm	200ml
03	15gm	200ml

Fig.02

4.2 Development & Treatment of Fresh Yeast Culture

Fresh cream yeast is grown in a controlled isolated environment. The steps for growing yeast are as follows:-

a) A **2 litre** plastic jar is selected. The jar is rinsed with boiling water containing detergent in order to wash off the dirts and impurities. Then it is sterilized with disinfectant in order to kill all unwanted microbial race and run overs. Then again it is rinsed with boiling water to remove traces of above cleansers. **The jar size is chosen as such in order to contain the rapid yeast culture take off consistently.**

N.B: The jar should be effectively heat resistant and made of high quality polymer to avoid unwanted chemical reactions during on going procedures.

b) Now about **120ml/1 cup** dechlorinated/potable/filtered or safe drinking water is poured into the jar. The water should be at least mineral "hard" in sense as yeast grows well in it.

N.B: Running tap water is strictly prohibited because of impurities and surplus microbial run.

c) Now **3 cups/360ml** of good fine grained flour is added into the water and vigorously agitated in order to aerate the mixture thoroughly. Sufficient aeration is a must as yeast cells perform aerobic respiration(0) and produce CO2 which is essential for bread starter. CO2 is noticed by rising and foaming of the broth rapidly at initial stages.

d) Now **150 gm** of organic washed grapes(0) are added into the broth. They are rinsed in running tap water to remove all dirts and impurities even traces of pesticides and harmful chemicals.

Fig.03

e) Knead the broth thoroughly with a highly sterilized disinfected stirrer to mix everything in equivalent distribution. This results in some more aeration. Crushing some of the grapes with the stirrer results in **"musting"(crushed vine solution containing particulate solids)** which savours out the sucrose(0) which is a potent yeast food.

f) Then the whole set up is covered but not sealed. Covering is done with a cotton cloth which exclusively permits the exit of CO2 but keep atmospheric gases at bay, it also allows further oxygen to thrive in when needed by yeast.

N.B: Using air tight lid might lead to breakage due to rapid production of CO2 by successful starter hence internal pressure is more than external one. Absence of free oxygen might also lead to fermentation(0,0) and putrefaction assisted by break down of flour proteins glutenin and gliadin to rotten gases like H2S, NH3 by microbes.

g) Now is the time that the set up is stored overnight at a sustainable warm place of **(21-36°C)/(70-85°F)** favourable for growth.(0)

N.B: Too much warm environment will kill the yeast cells where as too much cold place will retard growth rate.(0)

h) The initial growth occurs rapidly and the mixture doubles in the container with abrupt foaming and bubbling plus heat evolution since cellular respiration is exothermic. It takes **12 hours** to ascend the first growth. Then the set up is observed when it turns **pale brown** after further **12 hours** the colour is **caramel brown** which signifies complete utilization of food. The pH is measured by taking a bit of broth in a **petridish.** Yeast favours **4.5-5.0 pH(0,0)** level for optimum growth. **A pH range of 4.5-6.0 is best.(0)**

Fig.04 Pale Brown

Fig.05 Caramel Brown

N.B: If the broth appears to be blackish it is evident that yeast cells have completely run out of food and off flavour components has turned in due to abnormal oxidation of metals like Mn, Mg, Fe, Cu etc which yields colour on oxidised state(MnO, CuO, MgOH, Fe2O4), a conditioned termed putrefaction by microbes. The whole nine yards should be discarded without hesitation.

i) After **1 day again 120ml** of potable water and 360ml of flour is added into the culture and agitated like before. Again the set up is kept for further **24 hours** as yeast eats new food. The colour turns **tawny.**

Fig.06 Tawny Brown

j) Now the whole culture is discarded leaving **120 ml** into the jar and restore the discarded volume with 120 ml water and 3 cups flour freshly. Aeration with stirrer is done just slightly. The procedure is continued for 3 days unless the top of broth becomes **chrome yellowish.** In course of time the starters left on each intermediates ferments the sucrose(0) to pyruvic and lactic acid(0) with emission of other volatile organic compounds(VOC) like acetaldehyde, ethanol, isopropanol, butanol, acetates, 2-3 butanediol etc.(0) Hence the culture turns sour and acidic which is in turn immensely favourable for growth. Thus the term **"sourdough starter". Yeast cells stars 4.76(0) pH for their best replicative lifespan.(0)**

Fig.07 Chrome Yellowish

N.B: Too much pungent sour odour implies that fermentation is at peak and the culture would be jar of organic acids(0), pH would drastically fall to 2-3 range which is misnomer.(0) Buffering the resultant with Baking soda(NaHCO3) will not increase replicative lifespan of yeast(0) instead it would leaven the culture just like yeast to ferment more and release VOC(s).(0)

k) Eyes are kept on progress unless the sour smell is outrun by more bread like smell and the broth doubles between each intermediate feeding. Once it occurs the culture is ready to be refrigerated. The pH is again measured and adjusted by repeating steps **(i to k).**

l) Finally the whole set up is refrigerated by tightly covering the jar due to which yeast goes dormant and further growth is stunted. Feeding is done seldom once a week by discarding the broth to avoid overflow and can be kept for **months or years in this mode.**

Fig.08

4.3 Dough Preparation with Organic Ingredients & Yeast Starter

- **Control = 150 gm flour + 5gm fresh yeast broth + 5gm sugar**

Table 4.3: Formulation for dough preparation

Wt. of flour	Wt.of Amaranth selected	Wt. of Spices	Vol. of Red pigment	Vol. of Ascorbic acid	Wt. of Fresh Yeast Broth	Wt. Of Sugar
150 gm	5 gm	4 gm	20 ml	10 ml	5 gm	5 gm
		6 gm	20 ml	10 ml	5 gm	5 gm
		8 gm	20 ml	10 ml	5 gm	5 gm
150 gm	10 gm	4 gm	20 ml	10 ml	5 gm	5 gm
		6 gm	20 ml	10 ml	5 gm	5 gm
		8 gm	20 ml	10 ml	5 gm	5 gm
150 gm	15 gm	4 gm	20 ml	10 ml	5 gm	5 gm
		6 gm	20 ml	10 ml	5 gm	5 gm
		8 gm	20 ml	10 ml	5 gm	5 gm

a) **150 gm/1.5 cups flour** is taken in a highly sterile disinfected pot.

3 similar pots like this are set up.

Fig.09

N. B: The pots must be good enough to resist microwave oven heat and must be cleansed with detergent and disinfectant then washed thoroughly with distilled water.

b) **5 gm Amaranth leaves red pigment 20 ml extract** is added into each of the 3 pots consecutively.

c) **4gm, 6gm, 8gm spice mixtures** of**(Clove, Cardamom, Nutmeg, Cumin, Fenugreek, Cinnamon, Mustard, Aniseed, Mace, Black Pepper finely powdered)** mixed in ratio of **1:1:1:1:0.5:1:1:2:1:0.5** by weight for each of the above weights are added individually into the mixture of the 3 pots consecutively.

Fig.10

d) **5 gm fresh yeast broth** is added into each of the 3 pots consecutively.

Fig.11

e) **5 gm sugar** is also added into each of the 3 pots consecutively.

f) **10 ml ascorbic acid** as **dough conditioner** is also added into the mixture of each of them consecutively.

Fig.12

g) Then the whole mixture is kneaded, punched and tumbled manually in all 3 of them individually to evenly distribute the ingredients throughout.

h) Then the doughs prepared are kept into three sterilized cleansed containers with respective labels.

i) The steps (a-h) are repeated with 10 gm and 15 gm Amaranth leaves extract separately and another 2 sets each of 3 samples are prepared individually.

j) ****A control sample with only 150 gm flour, 5 gm fresh yeast broth and 5 gm sugar is prepared to centralize the basic statistic of the dough to avoid anonymous data statistics.**

4.4 Baking all samples to bread

The baking process has been staged as follows:-

Stage 1- Leavening with fresh cream yeast starter(Proofing) :

Dough is usually leavened by fresh bread yeasts starter, which ferment the sugars in the dough and produce mainly CO2 and alcohol(0). There is no growth or little growth during the **first 4-6 hours** after the yeast is added to the dough, but some growth

in **6-8 hours,** if that much time is allowed before baking and then a decline in growth in **10-12 hours.** Fermentation by the yeast begins as soon as the dough(sponge) is mixed and continues until the temperature of the oven inactivates the yeast enzymes. The professional baker adds a considerable amount of yeasts and has a comparatively short making time.

The **fermentative "conditioning"(0,0)** of the dough takes place when the flour proteins (gluten) mature and becomes elastic and springy and therefore capable of retaining a maximal amount of the carbon dioxide produced by the yeast. The conditioning results from the action on the gluten by **(i) proteolytic enzymes in the flour form the yeast, from the malt, or added otherwise and (ii) the reduction in pH by the acids added and formed.(0)** Dough conditioner sometimes called yeast food that is added **e.g ascorbic acid** to improve dough characteristics.(0,0)

Although the sugar in the flour plus that produced by the action of amylase on the flour may be enough for yeast fermentation, most formulas call for the addition of more sugars or of amylase-bearing malt. The rate of gas production by the yeasts is increased by adding **(i) more yeast (ii) sugar or amylase bearing malt and (iii) yeast food within limits (iv) a little bit of salt.(0)** It is decreased by the **(i) addition of salt (ii) the addition of too much yeast food and (iii) the use of too high or too low temperatures.** The main gas produced and to have the dough in such a conditioning that it will hold the gas at the right time is **CO2.**

In the sponge method of bread making, some of the ingredients are mixed at 23-24℃ and allowed to ferment to the desired maturity. Then the rest of the ingredients are added and the fermentation is continued until the dough is in the desired condition. In the straight-dough method of making, all the ingredients are mixed at 26-28℃. The fermentation room where the dough is held for almost of the leavening process, is usually held at about 27℃.(0)

Stage 2- The baking process :

Although the interior of the loaf does not quite reach 100℃ during baking, the heat serves to kill the yeasts inactivating their enzymes and those of the flour and malt, expands the gas present and set the structure of the loaf. Baking besides producing the appearance of the loaf also contributes desirable flavours. The yeast also drives off most of the alcohol and other volatile substances formed by the yeasts but contributes substances such as **furfurals, pyruvic acids and other aldehydes.** Often other organic compounds that add to the flavour. The most important change in bread during the baking procedure is **"gelatinization"** of starch. **"Set"** of bread results from this process, in which gluten gives structural support in the dough but starch supports the structure of baked bread.(0) Baking is done at variable temperatures ranging from **(30-50)℃/(86-122)˚F for near about 1.5-3 minutes (0,0)** depending on the size and type of dough prepared. Here at 30℃ for optimum 3 minutes baking is completed.

Fig.13

Stage 3- Flavour production(Leavening after baking with residual steam) :

Yeasts are reported to contribute to the flavour of bread through products released during the fermentation of the sugars during post baking leavening of **2 hours**.(0) Alcohol, acids, esters and aldehydes are products that add desirable flavours during first **45 minutes.** Most experts believe that bacteria growing in the dough can contribute the most of flavours. Too little time is allowed in the usual industrial leavening and working process for the bacteria to grow enough to appreciably affect the flavour. Most of the flavours in bread comes from the chemical reactions and the ingredients that occur such as **maillard browning(0)** during baking. If enough time about **2 hours** is given previous to baking for the growth of bacteria, they add to flavour, as do the yeasts to the lesser extent. Flavouring substances so developed during first **1 hour** after baking may include **alcohol, aldehydes and iso-alcohols and lactic, acetic and succinic acids and their esters.**

4.5 Procedure of Sensory Analysis

A total of 10 panel members are chosen randomly and subjected to evaluate the score of the respective test samples (each member is suggested to examine all 10 samples and put the grade point of all 5 attributes accordingly for each of them individually). The method is done stereotypically for each of 10 members.

N.B: Mean(X) = Points of (M1+M2+M3+M4……M10)/10
M1=panel member 1, M2=panel member 2……M10=panel member 10
***Control Sample(raw ingredients assembled without additives)**

V. RESULTS

5.0 Observations

- **Blanching tests' pigmentation statistics(each set for 5 gm, 10gm and 15gm Amaranth):**

Table 5.0: Test 01 with CH3CH2OH

Pigment intensity	Colour Code	Colour Profile
light	PMS 361	
moderate	PMS 364	
strong	PMS 357	

Table 5.0: Test 02 with Luke Warm D/W

Pigment intensity	Colour Code	Colour Profile
moderate	PMS 220	
light	PMS 214	
strong	PMS 229	

Table 5.0: Test 03 with 50% CH3CH2OH+50% Luke Warm D/W

Pigment intensity	Colour Code	Colour Profile
Deep	PMS (361+214)	
Dense	PMS (220+364)	
Dark	PMS (357+229)	

5.1 Results of Sensory Analysis

Sensory Evaluation calculated Mean(x):

Table 5.1: Mean(X) score of evaluated attributes of 10 panel members

Table:05/ Tests=10		**9-point Hedonic Scale Assessment : mean(X) score of 10 panel members**				
		for cross-ref. data statistics of sensory evaluation				
Parameters\ Test Samples	**Color (X1)**	**Flavour (X2)**	**Texture (X3)**	**Taste (X4)**	**overall acceptability (X5)**	**SCORE PROFILE:**
1	**4.7**	**4.2**	**4.9**	**4.9**	**4.8**	**1 = Dislike Extremely**
2	**4.2**	**4.2**	**6**	**4.5**	**4.6**	**2 = Dislike Very Much**
3	**5.6**	**4.6**	**5.3**	**5.2**	**4.9**	**3 = Dislike Moderately**
4	**4.4**	**4.8**	**4.8**	**4.9**	**5**	**4 = Dislike Slightly**
5	**3.9**	**2.8**	**4.5**	**2.4**	**3.8**	**5 = Neutral****
6	**4.4**	**4.7**	**4.8**	**4.1**	**4.8**	**6 = Like Slightly**
7	**4.5**	**5.4**	**4.9**	**4.5**	**4.9**	**7 = Like Moderately**
8	**4.5**	**5.7**	**4.8**	**5.1**	**5.2**	**8 = Like Very Much**
9	**4.6**	**5.4**	**4.9**	**5.5**	**5.2**	**9 = Like Extremely**
10*	**4.8**	**3.4**	**4.1**	**3.4**	**3.4**	

****Implies neither good nor bad**

Thus from the above grade point evaluations it is evident that the sample numbers 3 and 9 are likely to be the acceptable overall to some extent comparing to the other ones. Hence its inferable that these two samples could meet the required

qualitative features of marketability in every aspect of enhanced sensory, medicinal, shelf-life and flexible properties consistently rather than traditional commonly marketed bread.

- **The following are the graphical data plot charts for visual interpretations of the sensory statistics:-**

Fig.14 9-point hedonic mean score

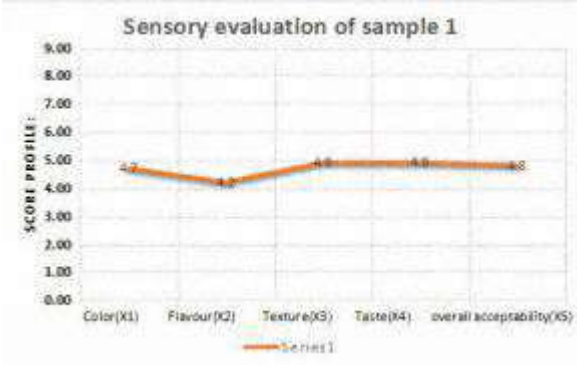

Fig.15

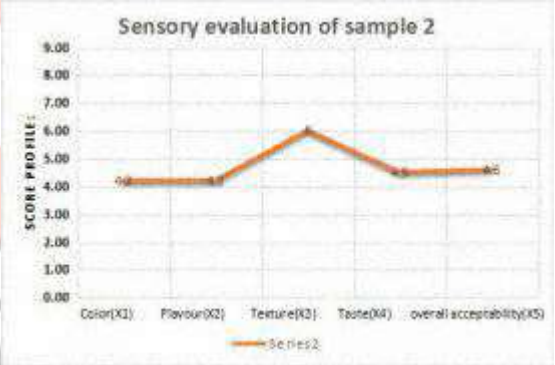

Fig.16

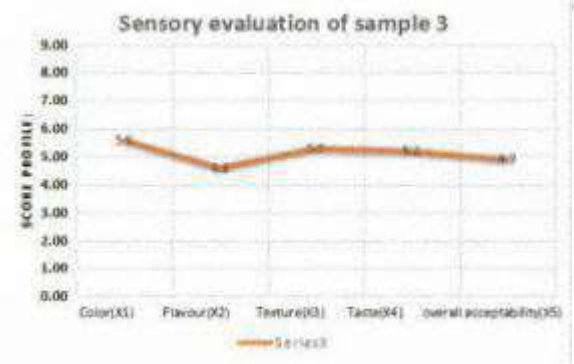

Fig.17

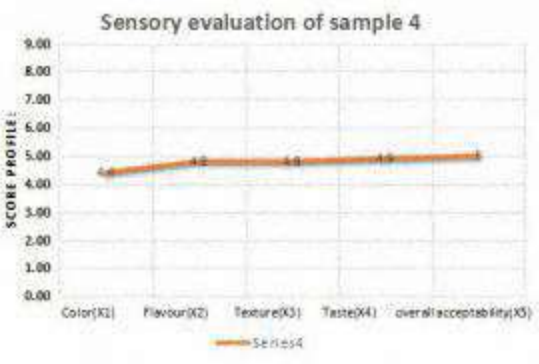

Fig.18

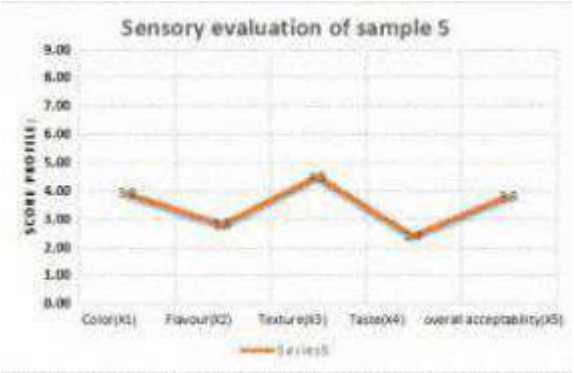

Fig.19

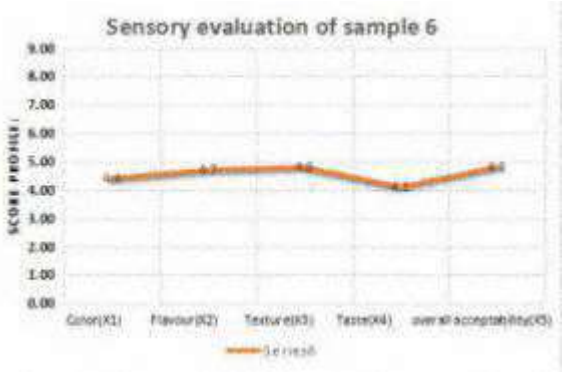

Fig.20

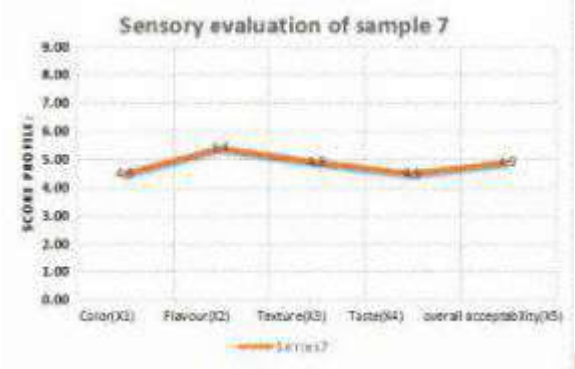

Fig.21

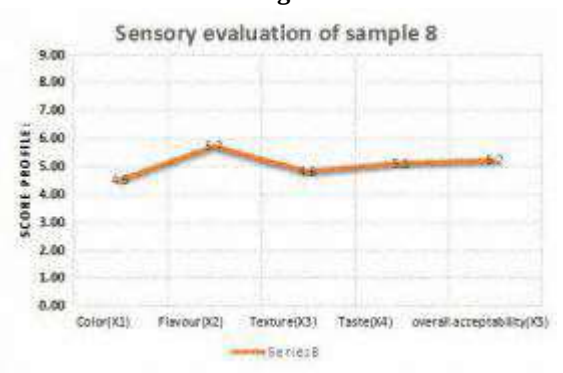

Fig.22

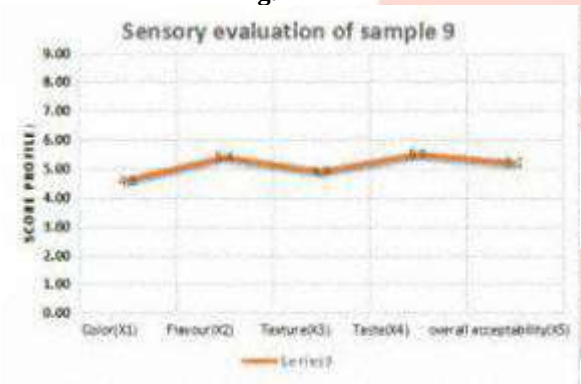

Fig.23

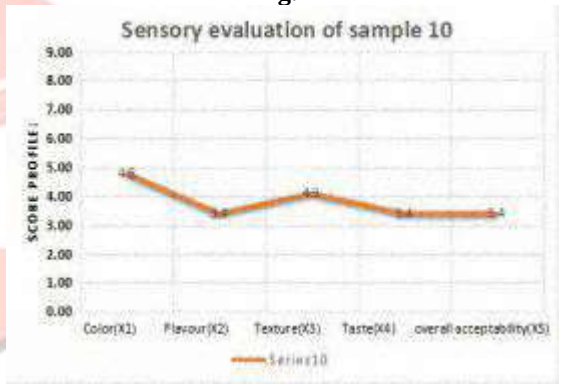

Fig.24

5.2 ANOVA Statistical Analysis

ANOVA Analysis between ten bread samples are as follows:-

Test Sample / Parameters	1	2	3	4	5	6	7	8	9	10
Color	4.7	4.2	5.6	4.4	3.9	4.4	4.5	4.5	4.6	4.8
Flavour	4.2	4.2	4.6	4.8	2.8	4.7	5.4	5.7	5.4	3.4
Texture	4.9	6	5.3	4.8	4.5	4.8	4.9	4.8	4.9	4.1
Taste	4.9	4.5	5.2	4.9	2.4	4.1	4.5	5.1	5.5	3.4
Overall acceptability	4.8	4.6	4.9	5	3.8	4.8	4.9	5.2	5.2	3.4

ANOVA : Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
1	5	23.5	4.7	0.085
2	5	23.5	4.7	0.56
3	5	25.6	5.12	0.147
4	5	23.9	4.78	0.052
5	5	17.4	3.48	0.737
6	5	22.8	4.56	0.093
7	5	24.2	4.84	0.138
8	5	25.3	5.06	0.203
9	5	25.6	5.12	0.137

10	5	19.1	3.82	0.392

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	13.62	9	1.51	5.95	3E-05	2.12
Within Groups	10.176	40	0.25			
Total	23.79	49				

ANOVA analysis between Test sample 1 and Test sample 2:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0	1	0	0	1	5.317655
Within Groups	2.58	8	0.3225			
Total	2.58	9				

ANOVA analysis between test sample 1 and test sample 3:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.441	1	0.441	3.80	0.09	5.32
Within Groups	0.928	8	0.116			
Total	1.369	9				

ANOVA analysis between test sample 1 and test sample 4:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.016	1	0.016	0.23	0.64	5.32
Within Groups	0.548	8	0.0685			
Total	0.564	9				

ANOVA analysis between test sample 1 and test sample 5:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.721	1	3.721	9.05	0.02	5.32
Within Groups	3.288	8	0.411			
Total	7.009	9				

ANOVA analysis between test sample 1 and test sample 6:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.049	1	0.049	0.55	0.48	5.32
Within Groups	0.712	8	0.089			
Total	0.761	9				

ANOVA analysis between test sample 1 and test sample 7:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.049	1	0.049	0.44	0.53	5.32
Within Groups	0.892	8	0.1115			
Total	0.941	9				

ANOVA analysis between test sample 1 and test sample 8:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.324	1	0.324	2.25	0.17	5.32
Within Groups	1.152	8	0.144			
Total	1.476	9				

ANOVA analysis between test sample 1 and test sample 9:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.441	1	0.441	3.97	0.08	5.32
Within Groups	0.888	8	0.111			
Total	1.329	9				

ANOVA analysis between test sample 1 and test sample 10:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.936	1	1.936	8.1174	0.021509	5.317655
Within Groups	1.908	8	0.2385			
Total	3.844	9				

ANOVA analysis between test sample 2 and test sample 3:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	30.276	1	30.276	4.944231	0.056865	5.317655
Within Groups	48.988	8	6.1235			
Total	79.264	9				

ANOVA analysis between test sample 2 and test sample 4:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	36.481	1	36.481	6.004115	0.039916	5.317655
Within Groups	48.608	8	6.076			
Total	85.089	9				

ANOVA analysis between test sample 2 and test sample 5:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	65.536	1	65.536	10.21049	0.0127	5.317655
Within Groups	51.348	8	6.4185			
Total	116.884	9				

ANOVA analysis between test sample 2 and test sample 6:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*

Between Groups	40.804	1	40.804	6.693021	0.032259	5.317655
Within Groups	48.772	8	6.0965			
Total	89.576	9				

ANOVA analysis between test sample 2 and test sample 7:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	35.344	1	35.344	5.776107	0.042951	5.317655
Within Groups	48.952	8	6.119			
Total	84.296	9				

ANOVA analysis between test sample 2 and test sample 8:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	31.329	1	31.329	5.092904	0.053996	5.317655
Within Groups	49.212	8	6.1515			
Total	80.541	9				

ANOVA analysis between test sample 2 and test sample 9:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	30.276	1	30.276	4.948272	0.056785	5.317655
Within Groups	48.948	8	6.1185			
Total	79.224	9				

ANOVA analysis between test sample 2 and test sample 10:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	57.121	1	57.121	9.145213	0.016456	5.317655
Within Groups	49.968	8	6.246			
Total	107.089	9				

ANOVA analysis between test sample 3 and test sample 4:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.289	1	0.289	2.904523	0.126732	5.317655
Within Groups	0.796	8	0.0995			
Total	1.085	9				

ANOVA analysis between test sample 3 and test sample 5:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.724	1	6.724	15.21267	0.004542	5.317655
Within Groups	3.536	8	0.442			
Total	10.26	9				

ANOVA analysis between test sample 3 and test sample 6:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*

Between Groups	0.784	1	0.784	6.533333	0.033855	5.317655
Within Groups	0.96	8	0.12			
Total	1.744	9				

ANOVA analysis between test sample 3 and test sample 7:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.196	1	0.196	1.375439	0.274615	5.317655
Within Groups	1.14	8	0.1425			
Total	1.336	9				

ANOVA analysis between test sample 3 and test sample 8:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.009	1	0.009	0.051429	0.826283	5.317655
Within Groups	1.4	8	0.175			
Total	1.409	9				

ANOVA analysis between test sample 3 and test sample 9:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.2216	1	2.2216	1.5615	1	5.317655
Within Groups	1.136	8	0.142			
Total	1.136	9				

ANOVA analysis between test sample 3 and test sample 10:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.225	1	4.225	15.67718	0.00418	5.317655
Within Groups	2.156	8	0.2695			
Total	6.381	9				

ANOVA analysis between test sample 4 and test sample 5:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.225	1	4.225	10.70976	0.011313	5.317655
Within Groups	3.156	8	0.3945			
Total	7.381	9				

ANOVA analysis between test samples 4 and test sample 6:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.121	1	0.121	1.668966	0.232466	5.317655
Within Groups	0.58	8	0.0725			
Total	0.701	9				

ANOVA analysis between test sample 4 and test sample 7:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.009	1	0.009	0.094737	0.7661	5.317655
Within Groups	0.76	8	0.095			
Total	0.769	9				

ANOVA analysis between test sample 4 and test sample 8:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.196	1	0.196	1.537255	0.25016	5.317655
Within Groups	1.02	8	0.1275			
Total	1.216	9				

ANOVA analysis between test sample 4 and test sample 9:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.289	1	0.289	3.058201	0.118454	5.317655
Within Groups	0.756	8	0.0945			
Total	1.045	9				

ANOVA analysis between test sample 4 and test sample 10:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.304	1	2.304	10.37838	0.012211	5.317655
Within Groups	1.776	8	0.222			
Total	4.08	9				

ANOVA analysis between test sample 5 and test sample 6:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.916	1	2.916	7.026506	0.029222	5.317655
Within Groups	3.32	8	0.415			
Total	6.236	9				

ANOVA analysis between test sample 5 and test sample 7:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.624	1	4.624	10.56914	0.011683	5.317655
Within Groups	3.5	8	0.4375			
Total	8.124	9				

ANOVA analysis between test sample 5 and test sample 8:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.241	1	6.241	13.27872	0.006551	5.317655
Within Groups	3.76	8	0.47			
Total	10.001	9				

ANOVA analysis between test sample 5 and test sample 9:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.724	1	6.724	15.38673	0.004402	5.317655
Within Groups	3.496	8	0.437			
Total	10.22	9				

ANOVA analysis between test sample 5 and test sample 10:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.289	1	0.289	0.511957	0.494643	5.317655
Within Groups	4.516	8	0.5645			
Total	4.805	9				

ANOVA analysis between test sample 6 and test sample 7:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.196	1	0.196	1.69697	0.228931	5.317655
Within Groups	0.924	8	0.1155			
Total	1.12	9				

ANOVA analysis between test sample 6 and test sample 8:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.625	1	0.625	4.222973	0.073933	5.317655
Within Groups	1.184	8	0.148			
Total	1.809	9				

ANOVA analysis between test sample 6 and test sample 9:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.784	1	0.784	6.817391	0.031081	5.317655
Within Groups	0.92	8	0.115			
Total	1.704	9				

ANOVA analysis between test sample 6 and test sample 10:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.369	1	1.369	5.645361	0.044825	5.317655
Within Groups	1.94	8	0.2425			
Total	3.309	9				

ANOVA analysis between test sample 7 and test sample 8:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.121	1	0.121	0.709677	0.424019	5.317655
Within Groups	1.364	8	0.1705			
Total	1.485	9				

ANOVA analysis between test sample 7 and test sample 9:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.196	1	0.196	1.425455	0.266706	5.317655
Within Groups	1.1	8	0.1375			
Total	1.296	9				

ANOVA analysis between test sample 7 and test sample 10:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.601	1	2.601	9.815094	0.013954	5.317655
Within Groups	2.12	8	0.265			
Total	4.721	9				

ANOVA analysis between test sample 8 and test sample 9:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.009	1	0.009	0.052941	0.823797	5.317655
Within Groups	1.36	8	0.17			
Total	1.369	9				

ANOVA analysis between test sample 8 and test sample 10:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.844	1	3.844	12.92101	0.007037	5.317655
Within Groups	2.38	8	0.2975			
Total	6.224	9				

ANOVA analysis between test sample 9 and test sample 10:

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.225	1	4.225	15.97	0.004	5.318
Within Groups	2.116	8	0.2645			
Total	6.341	9				

VI. DISCUSSION

- **Why Amaranth?**

Here the herb that has been our main concern of research is “Amaranth”. But the pertinent question is why? Let’s tip off the question in other sense>”Why not Amaranth?” Before moving on to the substantive answer if we scope on the wild greeneries in the outskirts of Indian states and union territories, we would find that enormous species of herbs are been cultivated as well as are born in the diversity abundantly. **Such as Coriander, Clove, Holy Basil, Indian Borage, Mint, Curry Leaves, Fenugreek, Spinach, Dill, Chilli, Malabar Spinach, Amaranth, Basella alba, Glinus oppisitifolius, Bottle gourd leaves, Pumpkin leaves, Green amaranth etc.** But the most spooky part is all of the above mentioned are edible in nature. Its awestruck to notice how Humans have resolved their evolution of hunger by searching these natural timber. Our citation consists of baking analysis with herbal extract so among those above mentioned species all of them have green(chlorophyll)CHONMg pigmentation in chloroplasts(chlorophyll containing membrane bound cytoplasmic organelles called plastids) as we know that **PS-1(700nm)** and **PS-2(680nm)** of visible wavelengths are maintained in order to shuttle the electron transport chain along with other pathways like **photorespiration(C2,C3,C4), calvin-benson cycle, hatch and slack pathway, CAM cycle, Hill reaction and Blackman or Dark reaction.** All these are essential for plant physiological regulation. But in sense green pigmentation has been stereotypic till date for research and analytical purposes. Exception is “Amaranth” which contains an extra pigmentation called **phycoerythrin(red)**. It has an open chain tetrapyrrole structure and is hot water soluble pigment. Henceforth red pigment has been a part of best thing since sliced bread which is reluctantly tried out at the drop of a hat.

- **Why Spices?**

Indian Spices have have been proven to be the best Therapeutical agents in any clinical prognosis unbeatably and irreversibly till date in entire world, they just have run out every other anti-ailment, anti-syndromic, anti-chronic, anti-endemic as well as anti-epidemic supplements that we are familiar with in today’s world. Nothing like Indian Spices or a concoction of them. They are the potential substitute of every of the other drugs that we consume routinely. "Every drug is lethal in its action" according to Cell Biology and sub-cellular anatomy. These spices can be consumed with healthy meals as the raw hot savoury matters most for delicacies. They have been well known for their anti-fungal and preservative behaviour against contaminative and unwanted inoculative microbes which decreases the staling properties, shelf life, sensory attributes and biochemistry of food

products hence spoilage.(0) They could even be consumed raw with only water after they are fried and grinded in definite proportions. The medicinal aspects of them have proved out to be the most effective and the best altogether.

➢ **Do(s) and Don't(s):** Since the herb is a sophisticated one so organic solvent or hot water **surfactant** or even **disinfectant** is not used to sanitize or surface sterilize the plant shoot. Due to the potential risk of **exo-osmotic shock** and cell burst release of pigments which could be fatal for pigment loss incurring, internal tissue damage and toxicity, only distilled water and sodium chloride solutions are used to sterilize the plant tissue.

➢ **Heuristic Selection of Solvent:** Among the above 3 Data Tables the later 2 are taken into consideration due to the fact that the discolouration which occurred by organic solvent **ethanol (CH3CH2OH)** is **green**. It is very evident that only the green pigment that is chlorophyll is dissolved into ethanol but not the **red pigment(phycoerythrin)**. An explicit question is why? The answer lies in the molecular structure of chlorophyll. Arnoff and Allen(1966) discovered 9 types of chlorophyll among which two, Chlorophyll a and Chlorophyll b are commonly studied. Chlorophyll a has an empirical formula of C55H72O5N4Mg while chlorophyll b has an empirical formula of C55H70O6N4Mg. The general molecular structure of chlorophyll has a **tetrapyrrole porphyrin head (15x15Å)** and a **long chain alcohol called phytol (20Å)**. The four pyrrole rings of porphyrin are linked by **methine bridges or "-CH=".** Each pyrrole is a five member ring with one nitrogen and four carbon. Nitrogen atom of each pyrrole ring lies inwardly. A non-ionic Mg atom lies in the centre of porphyrin ring with two covalent and two co-ordinate bonds.

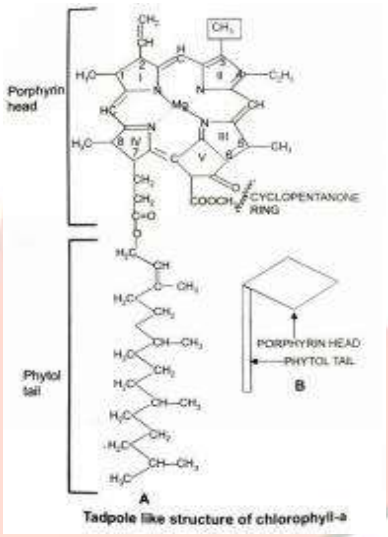

Fig.25

The outer carbons are numbered as 1-8 where a methyl group lies at carbon-1,3,5 and 8 while a vinyl group at carbon-2 and an ethyl group at carbon-3. Carbon-6 along with the next methine bridge forms a 5th isocyclic ring called cyclopentanone ring. Chlorophyll a has methyl group at carbon-3 and chlorophyll b has formyl group at carbon-3. Phytol ring is attached to the carbon-7 through propionic residue. **Phytol is C20H39OH**. It is pretty **non-polar** in nature hence insoluble in water but in organic solvent. Water molecules are polar in nature due to uneven distribution of charges. So water instead precipitates chlorophyll molecules.

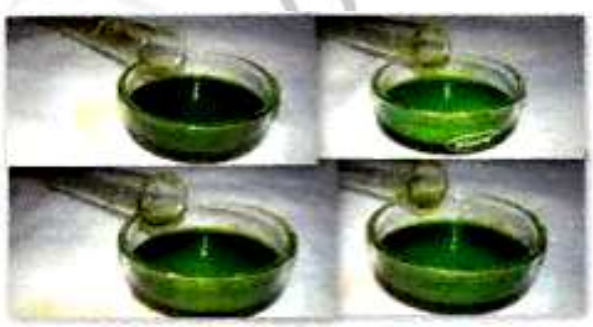

Fig.26

On the other hand the **phycoerythrin** structure is pretty much similar to chlorophyll but the **porphyrin tetrapyrrole is open chain linear** not enclosed into ring with a central metal atom unlike chlorophyll molecules. So its pretty **polar** in nature for which readily dissolves in polar solvent water but not in non-polar organic solvent ethanol.

Fig.27

Henceforth in third case we found that equal dissolution of chlorophyll as well as phycoerythrin occurs due to the fact that equal volume of ethanol and water are used as media together. The 2nd data statistics shows us that pink/red pigmentation appropriately occurred where as in the 3rd one greenish red/pinkish green pigmentation happened. Thus the later two are taken into account.

➢ **Introduction to Yeast:** Yeast is eukaryotic single-celled micro-organism belonging to the members of fungus kingdom. They vary from 3-4µm to about 40µm size. They reproduce mainly by asexual means by mitosis but some species do by asymmetric vegetative budding process or by binary fission. Yeasts are unicellular organisms they can be contrasted with molds which grow hyphae. Yeasts are dimorphic fungi(can take two life forms). There are about 1500 species of yeast out of which ***Saccharomyces cerevesiae*** is commonly used for best quality baking procedures. Yeasts perform cellular respiration in coordinated set of metabolic reactions in cell organelles that converts biochemical energy to nutrients and Andenosine tri-phosphate(ATP) with release of end products. Yeasts perform normal aerobic respiration summing up **glycolysis, cycle, oxidative decarboxylation, citric acid cycle, oxidative phosphorylation and electron transport chain for maintaining their life cycle.**

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + Energy(ATP) + heat$

(1)

$\Delta G = -2880KJ/686Kcal$ per mole of $C_6H_{12}O_6$

They can also survive in anaerobic conditions but are unfavourable for their proper growth. They are capable of carrying out both **exogenous (salvage)** and **endogenous(de novo)** pathways. Yeast cells ferment carbohydrates to higher alcohols and acids. The growth of these cells generate CO_2 which is very much essential for baking purposes. For years this art has been practised in large scale industrial economy. They have been now centralized as model organisms for cellular biological research purposes.

➢ **Standardizing Baking Protocol:** The following baking procedure has been done according to the above protocols of dough preparations. If all the ingredients are added in the dough in one go then the concoction would be a junked one. So in order to analyse the qualitative aspects the quantitative parameters should be varied to different assessments, the permutation of which will definitely give innumerable samples to be analysed individually. Hence to avoid unnecessary data statistics and over analysis of surplus samples, the dough preparation and baking procedure has been charted in a very solitary, compact, time saving and cost effective manner with standardized restricted amount of every ingredient in each sample. So we can Hit the nail right on its head.

➢ **Enzymatic activity within flour:** Amylase plays an important role on starch present which is revealed during baking process as such baking qualities of flour are influenced by the **diastatic(0)** or the distribution of amylases present. The diastatic activity not only influences the baking quality but also contributes to the extent to which the granules are affected by the diastase and the proportions of injured granules present.

Flour from sound wheat contains high amount of **β-amylase** and very little **α-amylase**. **β-amylase** converts **60% of damaged starch** into **maltose** which acts as a food for the yeast in the fermentation.(0) **β-limit dextran** is also produced which has a higher water holding capacity and the water absorption of the flour is increased by about **10% β-amylase** release is facilitated by the action of dilute salt solution.

As diastase is colloidal, it is not likely to diffuse through other colloids, such as gluten, very quickly. Severe grinding apparently increase the diastatic power of the flour because diastase is brought into intimate contact with the starch by such mechanical means and does not have to depend entirely on diffusion to reach the starch. Wheat itself contains a **proteinase** with **papain** like properties which is activated by the reduced **glutathione** and deactivated by oxidising agents, this enzyme first produces a clot and then liquifies the flour protein so that retention of gas by the dough is adversely affected i.e strength of the dough is decreased.

Another important enzyme is **α-amylase** which acts as the baking aid for better gas production.(0) It acts on damaged granules which are liquified and **dextrinized** so that in conjunction with the **β-amylase** it helps to bring about rapid and complete **saccharification** of the damaged granules mainly in the early stages of the fermentation.

Pure **xylanase** with single activity improves dough machinability.(0,0) Lipase has a gluten strengthening effect that results in more stable dough and **improved crumb structure** and a **maltogenic α-amylase** that has a unique **anti-staling effect.(0)**

Wheat and thus wheat flour contains endogenous and indigenous enzymes, mainly amylases. However, the level of amylase activity varies from on type of wheat to another. The amount of α-amylase in the non-germinated wheat or rye flours is negligible,. Therefore most bread flours must be supplemented with α-amylases, added in the form of malt flour or fungal enzymes. Fungal α-amylases are generally less thermostable, they are inactivated at temperatures near 65°C. Fungal α-amylases

act on the damaged starch content which can vary depending on wheat variety and milling conditions. Generally flour made from hard wheat contains more damaged starch than that from soft wheat. The α-amylases widely used in the baking industry can hydrolyse **amylose** and **amylopectin** to release soluble intermediate type **dextrins of DP2 and DP12**. The α-amylases provide fermentable sugar, which results in an increased volume, better crust colour, and improved flavour.(0) Due to hydrolysis of damaged starch a suitable dose of α-amylases results in a desirable dough softening.(0) However, extensive degradation of the damaged starch due to an over dose of α-amylase commonly leads to sticky dough.(0)

The level of indigenous and endogenous amylases will influence the starch **retrogradation** as well as the yeast action, thereby affecting properties of the final bread quality such as volume. Secondly, the level of damaged starch in the flour has an influence on the action of cereal and fungal α-amylase and therefore on the final quality of the bread. Although fungal α-amylases are effective in partially hydrolysing damaged starch as well as development of anti-staling effect due to their limited thermostability. They are mostly inactivated prior to the onset of starch gelatinization during baking when the bulk of the starch is available for modification.(0)

The bacterial α-amylases from ***Bacillus subtilis*** is able to inhibit anti-staling property by **hydrolysing glycosidic linkages** within the amorphous areas of **gelatinized** starch. Due to the high degree of thermostability, the enzymes can persist throughout baking and cooling and produce an excessive level of soluble dextrins.(0)

Maltogenic a-amylases has the thermostability between that of α-amylases and thermostable bacterial α-amylase. Therefore it is able to hydrolyse the glycosidic linkages of the gelatinized starch during the baking process, but it does not excessively degrade the starch because it is inactivated during the later stages of he baking process. A major advantage with maltogenic α-amylase is its tolerance to over dosing during the bread making process in the bakery. Maltogenic α-amylase does not affect dough rheological properties due to its low activity at a temperature under 35℃.

Lipase has recently been recognized as a strong dough conditioning enzyme. It shows excellent effects on bread performance. The loaf volume of various bread type increases significantly and crumb structure is more silky and uniform with its whiter appearance.

Wheat flour contains 1 to 1.5% lipids. These lipids are transferred into **monoglycerides** by the action of lipase, called hydrolysis. There is indeed limited formation of the **triglycerides** through this enzyme hydrolysis. The 1,3 specific lipase produces first and third position monoglycerides which are able to form complexes with starch, thus having a retarded effect on starch staling.

Addition of the fungal lipase to a flour dough does not significant change the rheological properties of the dough measured by both fasinograph and extensograph(0) with optimum water absorption. This is a good characteristic for bread making, because most bakers do not like major changes in the dough system. However gluten taken from lipase-treated wheat flour dough is significantly stronger and more elastic.(0)

Different oxidants such as ascorbic acid, citric acid, oxidases(0) are used in bread making. Oxidases are gaining increasing attention in the baking industry. Glucose oxidase, lipoxygenase, lysyl oxidase, sulfhydral oxidase, peroxidase, laccase and transglutaminase are good oxidising agents. Among these glucose oxidase has good oxidising effects that result in a stronger dough. Sometimes it is used with ascorbic acid for an excellent dough strengthener.(0)

- **Visual Interpretation of sensory evaluation:** In order to infer the flexibility, consistency and market assurance of our developed product with high esteem of qualitative inclinations, a sensory analysis has been made on 5 attributes like **"color", "flavour", "texture", "taste" and "overall Acceptability"(0,0,0,0)** of the prepared test samples. The **9-point Hedonic Scale assessment** shows the cross-reference data statistics of the attributes centralized by us with that of the panel members selected randomly and individually. The evaluated grades are been plotted in data charts showing the visual interpretation of the quantitative information collected hedonically in organized format. Quintessential depiction of rigorous scientific literature is a noteworthy agenda on graphical frame. The informed consent of respective grades are plotted graphically in order to visualize the variation between multiple datasets and relationship among the variable parameters. So also the error and uncertainty estimation of collected data can be analysed more easily from visual context and trend line of how data changes with time can also be conferred from the histograms.

- **Why ANOVA analysis is required?**

Analysis of variance (ANOVA) is a collection of statistical models and their associated procedures (such as "variation" among and between groups) used to analyze the differences among group means. ANOVA was developed by statistician and evolutionary biologist Ronald Fischer. In the ANOVA setting, the observed variance in a particular variable is partitioned into components attributable to different sources of variation. In its simplest form, ANOVA provides a statistical test of whether or not the means of several groups are equal, and therefore generalizes the t-test to more than two groups. ANOVA is useful for comparing (testing) three or more means (groups or variables) for statistical significance. It is conceptually similar to multiple two-sample t-tests, but is more conservative (results in less type I error) and is therefore suited to a wide range of practical problems.(0,0)

In simple words, ANOVA is a statistical method used to test differences between two or more means. In this experiment, we have done one way ANOVA analysis for ten bread samples.

VII. CONCLUSION

Been unfaltering to the modern covenant technology that has ameliorated overtime and is bestowed upon civilians to foster better livelihood in this **20th century 2nd millennium**, we the students shall provoke the commons in light of education regarding the pacific realms of proper knowledge. That realization must originate within one's own mind to be indulged with proper nutrition and healthy food which is **"A Living"** actually.

The term **"nutraceutical", a portmanteau** of the words **"nutrition"** and **"pharmaceutical"**, signifies a pharmaceutical grade and standardized nutrient that is regulated as dietary supplements and food additives.

Herbal food has a remarkable concoction of herbal extracts and raw substrates having a benchmark appetite to boost clinical pathways and bio-synthetic machineries in living bodies. Too much food does not necessarily mean more nutrition instead healthy and right choice of food in balanced quantity does. Necessity must not be an upper hand to "bite off more than can be chewed". The carbohydrates, lipids, proteins, minerals and vitamins that are consumed with food has significant role in physiological regulations in our body but there are even darker sides too. Hence the heroism of drugs evoked smoothly. Medicines that are our constant company in today's lifestyle costs an arm and a leg when subjected to dreadful consequences of food poisonings/infections. Thus utilizing natural organic extracts with food as medicine has given a brand new revolution to provide extra health benefits, in addition to the basic nutritional value. Depending on the jurisdiction, products may claim to prevent chronic diseases, improve health, delay the aging process, increase life expectancy, or support the structure and function of the body.

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