Use of Clove Carrot Mix in Cookies; A Way Towards Development of Low Caloric and High Fiber Cookies with Improved Organoleptic Properties

Noor-e-Huma[1], Dr. Nadia Akram[2] and Dr. Rabia Naz[3]

[1]Research Assistant, MPhil Food Science & Human Nutrition Department, Kinnaird College for Woman, Lahore, Pakistan
Email: noorehuma@live.com
[2,3] Assistant Professor, Food Science& Human Nutrition Department, Kinnaird College for Woman, Lahore, Pakistan
Email: nadia.akram@kinnaird.edu.pk, rabia.naz@kinnaird.edu.pk

Corresponding Author
Noor-e-Huma
93-Jail Road, GOR-I, Lahore, Pakistan
Email: noorehuma@live.com

Published: 31 July 2020

Cite this article: Huma, N., Akram, N., & Rabia Naz, R. (2020). Use of Clove Carrot Mix in Cookies; A Way Towards Development of Low Caloric and High Fiber Cookies with Improved Organoleptic Properties. *European International Journal of Science and Technology*, 9(7), 1-15.

Abstract
Cookies are an easy to go snacks with an increasing demand owing to their ease of consumption in this era of changed dietary patterns and sedentary life style. The effect of clove substitution in carrot flour cookie was assessed. The carrot flour being a good source of high fiber and low-calorie ingredient was used to assess the organoleptic properties of the cookies. The composite flour consists of carrot flour and white flour in two different ratios as; 50:50% (CF1) and 70:30 % (CF2), respectively with 2.5g of clove added to each. The best selected ratio after sensory evaluation was first analyzed for its nutritional composition, standardized for a replicable model and tested for the proximate estimation. Proximate analysis revealed a considerable change in crude protein (6.60% to 9.46%), crude fiber (4.10% to 5.80%). Clove incorporation improved the sensory quality of composite flour cookies and can open new opportunities for development of functional products with added health benefits specifically the snack items like cookies.

Keywords: Clove, nutritional composition, proximate analysis, therapeutic value, aromatic plant, beta-carotene, carrot flour.

1. Introduction

In the present world, nutraceuticals have gained great attention owing to their nutritional properties, safety as well as therapeutic effects. The term "Nutraceutical" refers to a substance that is derived from a food source that consist of dietary benefit of a disease and the already present base nutritional values present in that food. (Zeisel, 2018). [1]Development of snack item with less energy density are on high rise and carrot pomace can be good source of it in this regard as it may not only help in waste utilization but also improve nutritional quality of cookies. Besides, spices are rich source of antioxidants and amongst them, clove is one. Its usage is limited to the ethnic foods only, but can be added in snack items such as cookies, so need to look for ways by which clove can become part of such type of snacks. About 15-20% essential oil is present in a good quality clove bud with three most prominent volatile essential oils. The plant possesses one of the richest sources of phenolic compounds such as eugenol, eugenol acetate and gallic acid. (German, 2017).

Cloves are commercially harvested in India, Indonesia, Pakistan, Tanzania and Sri Lanka. It is used for medicinal purpose like different therapeutic and dental complaints in China and Western countries (E W Riptanti, 2018). The plant is also used to treat various gastrointestinal disorders such as diarrhea, vomiting, nausea, cough, dyspepsia, flatulence, stomach distention, gastrointestinal spasms along with uterine contractions and stimulate nerves. (Mahmoud S. M. Mohamed, 2018). The essential oils derived from the plant not only contribute to aromas and flavor enhancers but also prevent several diseases caused by free radicals such as cancer.

Clove oil has been termed as "Generally Regarded as Safe" substance by the United States Food and Drug Administration. (Jian-Guo Xu, 2016). Eugenol and eugenol acetate are part of clove and act as antioxidants. Eugenol dose dependently binds to membranes thus, stabilizing them and protecting them against free radicals and invading agents. Compound **3** inhibits lipid oxidation and helps to limit structural changes to various tissues, such as the heart, kidney, and liver.

[1] According to Food and Drug Administration, a product can bear the structure of a functional claim if the claim is derived from the nutritive value itself. However, if the claim describes the structure of the function that is not related to the nutritive value of the product, the claim can only be made if the product complies to the marketing requirements as a dietary supplement or drug. (Ross, 2000)

Compound **3** inhibits histamine release from mast cells to reduce hypersensitivity besides having anti-anaphylactic and antispasmodic properties. Cloves inhibit oxidative tissue damage and cataract formation in the eye lens of rats (Daniel Pereira Bezerra, 2017).

Three kinds of volatile liquid fats are yielded from clove, , oil extracted from the leaves, the stem and the buds. These are different from each other in terms of yield and quality. 1-Phenyethyl acetate is present in the clove bud essential oil and also in the grounded clove buds. (Klaus Gassenmeier, 2017). It is commonly identified as a flavoring agent.

About 15-20% essential oil is present in a good quality clove bud. The oil is ruled by β-caryophyllene (11.54%), eugenol acetate (1.76%), caryophyllene oxide (4.29%) and lastly eugenol that is present in highest amounts(76.23%), respectively. These makes up the 99% of oil. (Jian-Guo Xu, 2016). β-caryophyllene, that was the artefact of the distillation, has been reported to be a constituent of bud oil. Other essential constituents of clove oil are acetyl eugenol, vanillin crategolic acid tannins such as bicornin[2], methylsalicylate, gallototannic acid, flavonoidseugenin, rhamnetin, kaempferol, and eugenitin, triterpenoids such as oleanolic acid, campesterol and stigmasterol, and several sesquiterpenes. (Md. Azir Uddin, 2017).Eugenol (EUG,4-allyl-1-hydroxy-2-methoxybenzene) a natural phenolic compound has been very well investigated for its pharmacological effects, and studies have shown it to possess significant antioxidant, anti-inflammatory, cancer-preventive, analgesic, and local anesthetic activity. (Liang-Liang Zhang, 2017)

Carrots (Daucus carota) is a traditional local grown vegetable in Pakistan.It is popular partly due to its freshness and due to the bulk, it provides in meals/salads. Carrots contain 50% β-carotene and could successfully be used as a supplement in cakes, cookies, dough and other types of functional products. The nutritional content of carrot is reported to be high especially amino acids, fatty acids and minerals. The presence of high amount ofβ-carotene in carrots makes it inhibit free radical scavenger, anti-mutagenic and immunity booster. Carrots are also a prominent source of calcium pectate and exceptional pectin fiber that has cholesterol reducing properties, anti-carcinogenic, anti-atherosclerotic and anti-hypertensive properties.(J. A. Ayo, 2017).

For the current study, clove was selected as the functional ingredient to be in cooperated in carrot flour cookies. Cookies were selected as the experiment product because of its popularity among the household consumption as a favorite tea time snack(Kehinde, 2017). Clove is one of the many nutraceuticals available to human kind.

[2] Bicornin is an ellagitannin found in the cloves and has a luteic acid group.

2. Material & Method

The current study was an experimental research which is designed to replace the all-purpose flour cookies with composite flour (CF) using carrot flour (*Daucus carota subsp. Sativusbelonging to Apiaceae family*)with addition of clove(*Syzygium aromaticum*) as a bioactive compound. The study is quantitative and conducted in a sequential procedure, involving product development, product's sensory evaluation, product standardization, nutritional evaluation followed by proximate analysis. The product that was accepted by the sensory evaluators was tested for the proximate analysis as experimental product. The proximate was conducted for a clove-enriched, experimental "E" cookie and clove-excluded control "C" cookie, in Biotechnology Lab, using standard protocols and procedure of AOAC Methods. (AOAC Scientific Solutions, Standards & Methods, n.d.).

2.1 Product Development

Treatment plan for the composite flour (table 1 here)

2.1.1 Selection of Product

Carrot Flour was selected as the base ingredient for cookie. And, clove was added as a bioactive ingredient to the cookies. The experiment was done on cookies being the popular snack food by children and adults (Kehinde, 2017). Fresh carrots and cloves were purchased from the local market of Lahore city, Punjab, Pakistan.

2.1.2 Preparation of Ingredients

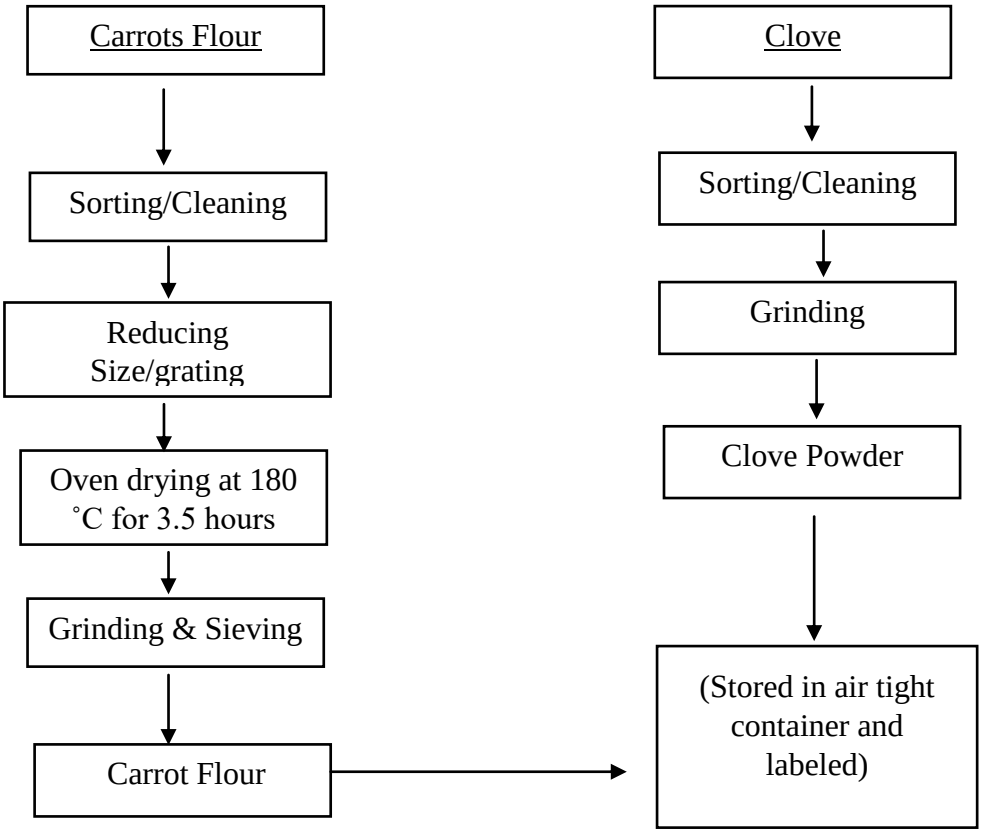

Figure 1: Flow Diagram for the Preparation of Ingredient

2.1.3 Development of Carrot Flour (figure 6 here)

2.1.4 Preparation of Cookies

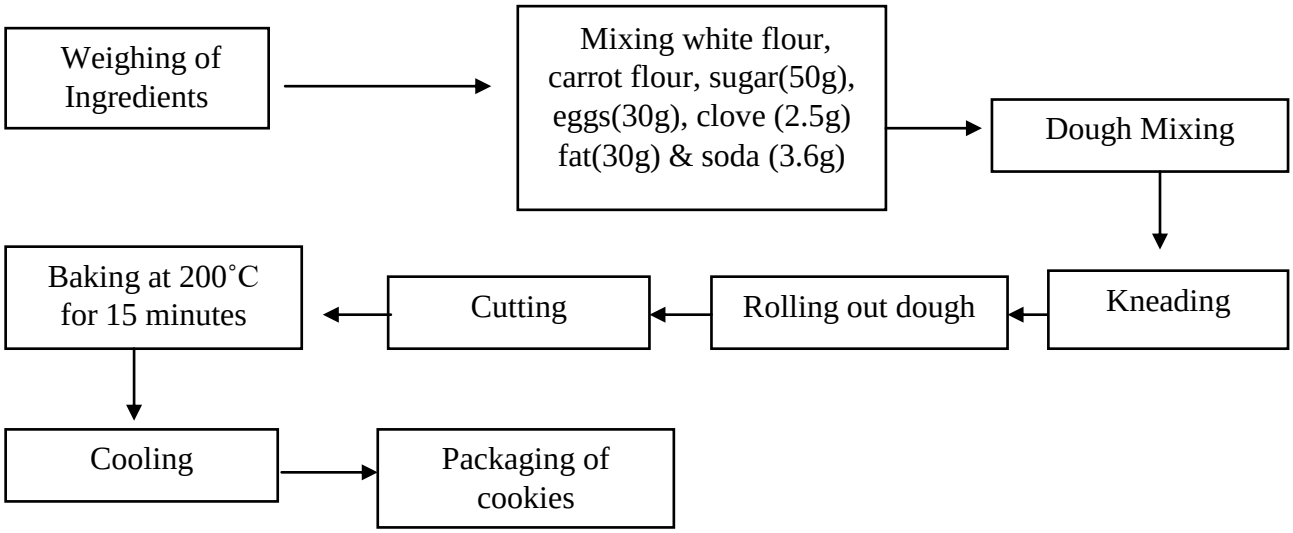

Figure 2: Flow Diagram for the Preparation of Cookies

2.2Sensory Evaluation and Standardization

The sensory evaluation of the product was carried out by 8trained panelists were selected from the faculty of Food Science and Human Nutrition department Kinnaird College for Women – Lahore, Pakistan, based on their familiarity with the cookies. The cookies were appropriately coded as "A" and "B" of the same size. The panelist rinsed their mouth with fresh water after tasting of the first sample, before moving onto the next sample. They were not allowed to make comments during evaluation to avoid biasness. Sensory evaluation was based on a 9-point hedonic scale with 1-Extremely Dislike to 9-Extremely Like. The product food characteristics were flavor, aroma, color, texture and overall acceptability. The accepted product was standardized for a replicable model and all the changes provided by the panelist, were incorporated and a standard recipe for clove enriched carrot cookie was developed.

2.3 Nutritional Value

The nutritional contents of the most acceptable cookies were analyzed. The analyses included the determination of energy, protein, amino acids, carbohydrate, fat, vitamins and mineral contents. These analyses were performed according to the procedures described in Official Methods of Analyses of the Association of the Official Chemists. (AOAC Scientific Solutions, Standards & Methods, n.d.)

2.4 Proximate Analysis for cookies

Proximate analysis was conducted in the University of Veterinary Sciences Lahore (UVAS) Pakistan, for the control product (Carrot flour cookies without clove) and the experimental product (Carrot flour cookies with clove). The experimental product chosen for proximate was based on the most preferred between CF1 & CF2 product during the sensory evaluation. Proximate parameters

(carbohydrate, crude fats, crude protein, crude fiber and ash) of the clove were determined using the Association of Official Analytical Chemistry Method(AOAC Scientific Solutions, Standards & Methods, n.d.) in an automated machine in (UVAS). Calories were calculated for the control and experimental group using the formula. Crude protein was calculated using the Total Kjeldahl method. The NFE was calculated using the equation given below:

NFE= [100-% (moisture +crude protein +crude fat +ash +crude fiber)]

(AOAC Scientific Solutions, Standards & Methods, n.d.)

2.5 Data Analysis

For the compilation of data, Microsoft Excel (2013) was used.

3. Results and Discussion

The carrot cookies enriched with clove were developed, nutritionally evaluated, standardized, sensory assessed for their sensory parameters and tested for proximate analysis. "CF1" represents 50:50 ratio while "CF2" represents 70:30 ration, the former being the carrot flour and the latter being the all-purpose flour, respectively. Standardization was done thrice in accordance to panelist's suggestions. Any suggestion made first time were incorporated in the next attempt to obtain the standardized recipe that can be replicated.

The sensory characteristics of cookies upon array of parameters were evaluated on the basis of flavor, aroma, color and texture and are shown in figure 3. The sensation of flavor perceived in the mouth and throat on contact with pieces of cookies was evaluated and the results are shown. CF2 cookies scored more in terms of sweetness (5:7) and richness (7:7). This is particularly due to the sweetness of the carrot flour present in greater quantity in CF2. The bitterness of CF2 was greater (5:7) owing to the presence of clove in the cookies. Although the clove quantity for both the cookies were same but the aroma was pronounced in terms of spicy-ness (4:6) and caramelly-ness (6:8) in CF2 owing to the presence of clove in cookies. The intensity or saturation of the color displayed not much diversion. Colors of the cookies were analyzed upon darkness, lightness and neutrality. CF2 were darker than CF1 scoring 4:6. This is particularly due to the presence of carrot flour in cookies which resulted in darker tone of CF2. The cloves were again present in equal quantity in both the cookies. The results of the texture appearance and mouth feel of the cookies was also analyzed. The addition of the clove to the cookies containing carrot flour did not much affect both the texture including the crunchiness and softness of the cookies, hence scoring 7:8 for both the parameters. While, crumbliness of the cookies scored 6:8.

Figure 4, shows the cookies with overall acceptability score. CF2 had greater degree of acceptance than CF1. Similar records were recorded in the results of the research by (Omachi .D.O, 2017) where cookies with more carrot flour and 2.5g of clove was more acceptable as compared to other treatments. It is evident in both the researches that if the composition of carrot flour increases, it is preferred by the panelists. It is also obvious from the results that supplementation significantly affected the overall acceptability of cookies. The maximum score recorded was 8.0 prepared from CF2 composite flour.

Cookies having higher sensory score were then standardized thrice to get the best response of all sensory attributes. The results are shown in figure 5of the three tests done for the standardization of clove enriched carrot cookies, in terms of their flavor, aroma, texture, color, taste and overall acceptability. The purpose of the standardization is to obtain a replication model for the study.

(Venkata Satish Kuchi, 2017). Any changes made by the panelist in first sensory evaluation were incorporated in the production before the second sensory evaluation. Hence, a standardized and replicable model was developed for future research and assistance.

The nutritional composition of a 3.5oz carrot cookie as control and 3.5oz carrot cookie containing 2.5g of clove as experimental is shown in table 2. The addition of clove in the cookies had a considerable increase in fiber, protein, folate and niacin. The manganese content raised from 4% to 100% having a very prominent contribution, proving that clove enriched carrot cookies are a good source of manganese and can contribute in bone health, regulation of blood glucose levels and play a part being an antioxidant.

Table 3records the proximate analysis of the approved biscuits during sensory evaluation and the control biscuit which is without the addition of clove. The moisture content of the cookies increased from 13.6% to 18.20% owing to the high moisture content of carrot flour. Similar results were reported in the study conducted by (Joel Ndife, 2014) where moisture content of carrot flour was higher than the all-purpose flour. this moisture range could be a demerit as most microorganisms can thrive and survive in this moisture, leading to spoilage and deterioration. There was a significant increase in the protein content of the cookies from 6.60% to 9.46%. The increase is due to the substitution of clove in cookies. Other studies about incorporation of clove into food products have also reported an increase in protein content in cookies owing to the supplementation of clove (Neveen Fahmy Mohamed Agamy. Neveen Ahmed Alwardany, 2015). The result implies that the cookie samples were high in protein content and could be used as alternative protein source in protein deficiency among children who are the major consumers. The fat content increased from 12.15% to 13.50% in the cookies produced from carrot flour and enriched with clove owing to the aromatic oils of the clove. This will have a considerable effect on the shelf stability of cookies. The ash content of the cookies increased a little by 3.14 to 3.36% due to the presence of clove. The crude fiber showed an increase in the clove enriched cookies than an un-enriched one from 4.10% to 5.80% as both carrots and clove contain a considerable amount of fiber. The fiber content increased with the addition of carrot flour which are good sources of fiber. Crude fiber is known to aid the digestive system of human. This is an advantage as it helps in bowel movement and easy digestibility. Similar results were reported in the study by (Joel Ndife, 2014) where the use of clove was used to increase the fiber content of the food product. The ash content was also enhanced with clove enrichment in cookies from 3.14% to 3.36% because cloves are naturally high in ash. Ash is a non- organic compound containing mineral content of food and nutritionally it aids in the metabolism of other organic compounds such as fat and carbohydrate. Beta carotene level was constant for both control and experimental product; 8.332mg per 3.5oz of one cookie.

The increased fiber content of cookies will have several health benefits as it will aid in digestion and reduce the burden in colon and affect the gut health preventing from constipation. The crude fiber content of cookies was within the range of recommendation of not more than 6g/day and other nonabsorbable carbohydrates per 100g dry weight(Hsi-YangTang, 2020). The protein content was also higher with the addition of clove in the experimental product and this will help in building degenerative tissues, muscles and hormones of the body. Proteins are the building blocks of the body necessary for growth, repair and maintenance(Ignacio Echeverría, 2016).

4. Conclusion

Clove is a spice that contains bioactive compounds which provide therapeutic roles in cancer prevention, diabetes management, bone health, heart health, promote gut health and reducing oxidative stress. Cloves were incorporated in carrot cookies with two different carrot flour ratios, 50:50% and 70:30%. The Clove enriched carrot cookies had acceptable aroma, texture, color, taste and overall acceptability. Enriched cookies with clove were found to be nutritionally superior, owing to high protein and fiber content. Thus, the clove enriched cookies can conveniently be regarded as complete whole snack ideal for tea times. However, further research work should be focused on the shelf stability o of the enriched cookies considering the high lipid content owing to aromatic oils of clove which can make the cookies prone to rancidity.

5. References

[1] Anees Ahmed Khalil, U. u. (2017). Essential oil eugenol: sources, extraction techniques and nutraceutical perspectives. *Royal Society of Chemistry*, 32669-32681.

[2] *AOAC Scientific Solutions, Standards & Methods*. (n.d.). Retrieved from aoac.org/: https://www.aoac.org/

[3] Arora M, S. S. (2015). PROCESS AND STANDARDIZATION OF PULSE BASED READY TO COOK NUTRITIOUS MIXTURE. *International Journal of Technical Research and Applications*, 97-99.

[4] Author linkHsiaolingWang, N. W. (2016). Protein Nitrogen Determination by Kjeldahl Digestion and Ion Chromatography. *Journal of Pharmaceutical Sciences*, 1851-1857.

[5] Daniel Pereira Bezerra, G. C. (2017). The Dual Antioxidant/Prooxidant Effect of Eugenol and Its Action in Cancer Development and Treatment. *Nutrients*, 1367.

[6] E W Riptanti, A. Q. (2018). Revitalization of cloves cultivation in Central Java, Indonesia. *The 1st International Conference on Environmental Sciences (ICES2018).* West Sumatra, Indonesia: IOP Publishing Ltd.

[7] German, C. J. (2017). Phytochemicals: nutraceuticals and human health. *Journal of the Science of Food and Agriculture*.

[8] Hsiaoling Wang, N. P. (2016). Protein Nitrogen Determination by Kjeldahl Digestion and Ion Chromatography. *Journal of Pharmaceutical Sciences*, 1851-1857.

[9] Hsi-YangTang, Z. K. (2020). Dietary fiber-based colon-targeted delivery systems for polyphenols. *Trends in Food Science & Technology*.

[10] Ignacio Echeverría, M. E.-C.-G. (2016). Structure, Functionality, and Active Release of Nanoclay–Soy Protein Films Affected by Clove Essential Oil. *Food and Bioprocess Technology*, 937–1950.

[11] J. A. Ayo, F. E. (2017). Physicochemical, Phytochemical and Sensory Evaluation of Acha-Carrot Flours Blend Biscuit. *Current Journal of Applied Sciences and Technology*, 1-15.

[12] Jian-Guo Xu, T. L.-P.-M. (2016). Chemical Composition, Antibacterial Properties and Mechanism of Action of Essential Oil from Clove Buds against Staphylococcus aureus. *Molecules*, 1194.

[13] Joel Ndife, F. K. (2014). PRODUCTION AND QUALITY ASSESSMENT OF ENRICHED COOKIES FROM WHOLE WHEAT AND FULL FAT SOYA. *European Journal of Food Science and Technology*, 19- 28.

[14] Kehinde, O. G. (2017). Quality Evaluation of Cookies from Under-Utilized Crop Source Blends. *American Journal of Food, Nutrition and Health*, 1-8.

[15] kizi, X. T. (2018). Convective drying of red carrots. *Proceedings of the ICECRS*.

[16] Klaus Gassenmeier, H. S. (2017). Unequivocal Identification of 1-Phenylethyl Acetate in Clove Buds (syzygium aromaticum and Clove Essential Oil. *Qualitative Analysis of Food Products*, 46.

[17] Liang-Liang Zhang, L.-F. Z.-G.-P. (2017). Comparison study on antioxidant, DNA damage protective and antibacterial activities of eugenol and isoeugenol against several foodborne pathogens. *Food & Nutrition Research* .

[18] Luis EduardoGarcia-Amezquita, V. T.-O.-O.-S.-C. (2018). Differences in the dietary fiber content of fruits and their by-products quantified by conventional and integrated AOAC official methodologies. *Journal of Food Composition and Analysis*, 77-85.

[19] Mahmoud S. M. Mohamed, A. A. (2018). Potential Alternative Treatment of Ocular Bacterial Infections by Oil Derived from Syzygium aromaticum Flower (Clove). *Current Eye Research*, 873-881.

[20] María E. HidalgoI, C. D. (2012). Antioxidant capacity of eugenol derivatives. *Química Nova*.

[21] Md. Azir Uddin, M. S. (2017). STUDY OF CHEMICAL COMPOSITION AND MEDICINAL PROPERTIES OF VOLATILE. *International Journal of Pharmaceutical Sciences and Research*, 895-899.

[22] Navneet Kumar, B. C. (2010). Development and Characterization of Extruded Product Using Carrot Pomace and Rice Flour. *International Journal of Food Engineering* .

[23] Neveen Fahmy Mohamed Agamy. Neveen Ahmed Alwardany, N. M. (2015). Effect of adding cinnamon, clove and vanilla on reducing the formation of acrylamide in some bakery products. *Journal of Research in Science and and Specific Arts*.

[24] Omachi .D.O, Y. .. (2017). Physico-chemical, sensory and microbiological assessment of millet based cookies improved with cashew nuts (Anarcadium Occidentale), carrot flour (Daucus Carota). . *AMERICAN JOURNAL OF FOOD AND NUTRITION*.

[25] Ross, S. (2000). Functional foods: the Food and Drug Administration perspective. *American journal of Clinical Nutrition*, 1735S–1738S.

[26] Samuel Wycoff, K. W. (2018). Effects of Clove Oil (Eugenol) on Proprioceptive Neurons, Heart Rate, and Behavior in Model Crustaceans. *Biology Faculty Publications*, 1-21.

[27] Shahzad HUSSAIN, F. M. (2006). Physical and Sensoric Attributes of Flaxseed Flour. *Turkish Journal of Biology*, 87-92.

[28] Shetty, P. G. (2018). Use of natural products for oral hygiene maintenance: revisiting traditional medicine. *Journal of Complementary and Integrative Medicine*.

[29] Venkata Satish Kuchi, J. K. (2017). Standardization of Recipe for Banana Burfi. *Advances in Bioresearch*, 270-274.

[30] Zeisel, S. H. (2018). Regulation of "Nutraceuticals". *Science*, 1853-1855.

6. Tables and Figures

Composite Flour	Carrot Flour	All PurposeFlour
CF1	50%	50%
CF2	70%	30%

Table 1: Treatment Plan for the Composite Flour

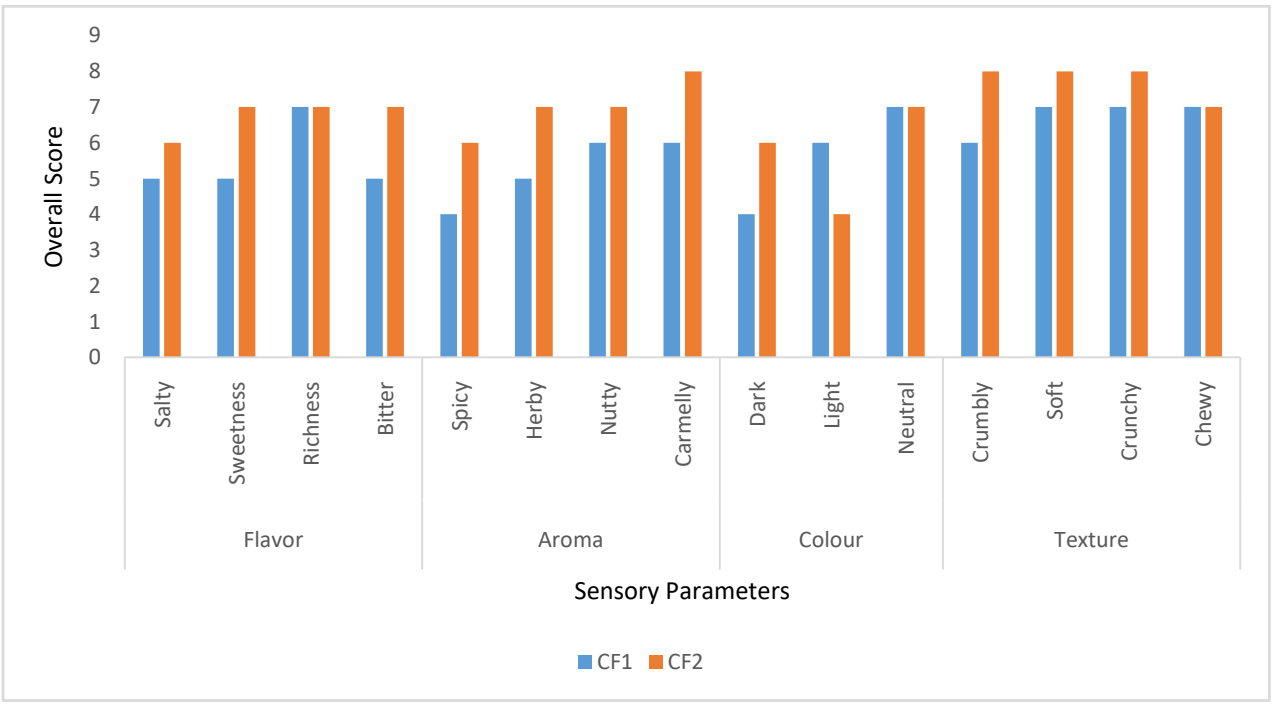

Figure 3: Sensory Evaluation of Clove Enriched Carrot Cookies

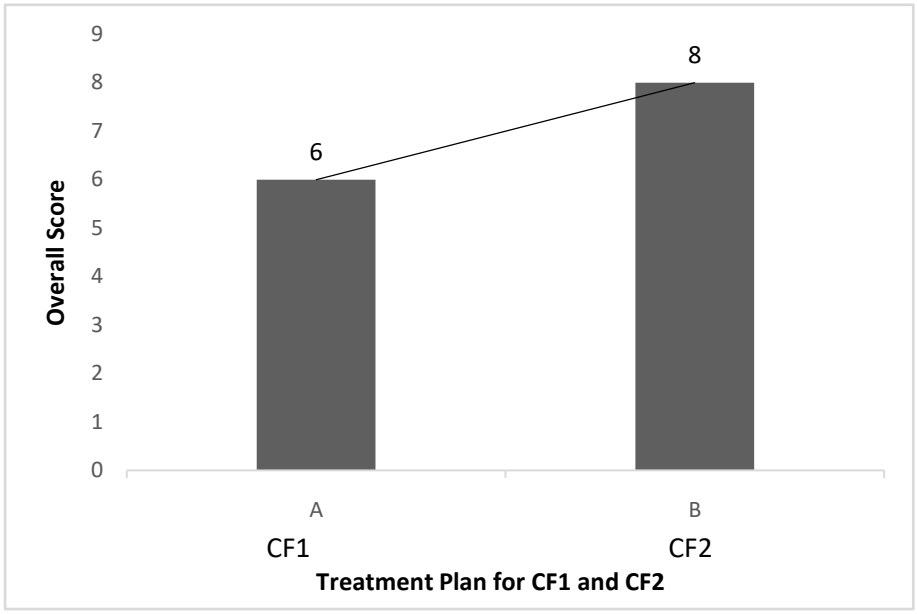

Figure 4: Overall Acceptability of Clove Enriched Carrot Cookies

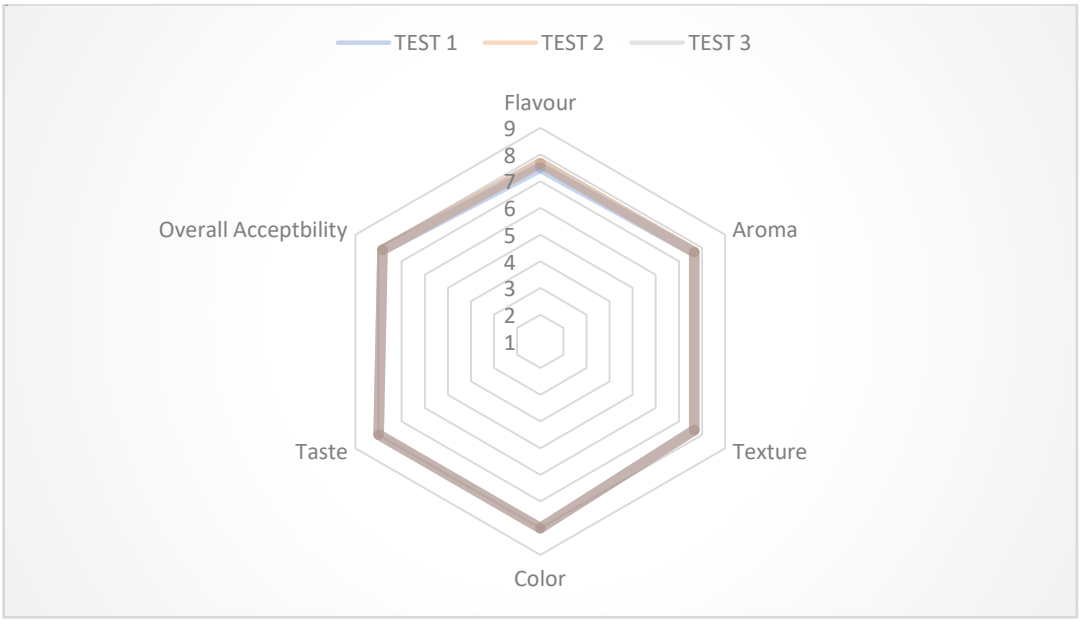

Figure 5: Standardization of Clove Enriched Carrot Cookies

Table 2: Nutritional Value for Clove EnrichedCarrot Cookies

Composition	Amount per Cookies-Control	Amount per Cookie-Experimental
Calorie	110.9	117.9
Carbohydrates	14.1 g	15.5g
Dietary fiber	0.5 g	1g
sugars	0.4 g	0.45g
fats	5.6 g	5.9g
Saturated	0.6 g	0.7g
polyunsaturated	3.1 g	3.35g
monounsaturated	1.6 g	1.65g
proteins	1.4 g	1.65g
Vitamin A	23.4 %	23.6%
Vitamin B-6	0.9 %	1.5%
Vitamin B-12	0.1 %	0.1%
Vitamin C	0.8 %	3.8%
Vitamin D	0.0 %	0.0%
Vitamin E	0.2 %	1.2%
Calcium	2.8 %	4.1%
Copper	1.0 %	1.3%
Folate	5.0 %	5.75%
Iron	3.1 %	4.1%
Magnesium	0.9 %	2.4%
Manganese	4.3 %	100%
Niacin	3.4 %	3.4%
Pantothenic Acid	0.7 %	0.7%
Phosphorous	2.3 %	2.6%
Riboflavin	3.5 %	3.8%
Selenium	4.9 %	5.2%
Thiamin	5.6 %	5.6%
Zinc	0.7 %	0.7%
Beta Carotene	8.332 mg	8.332mg

Table 3: Proximate Analysis of Clove Enriched Carrot Cookies

No:	Parameters	Control	Experimental
1.	Moisture (%)	13.6	18.20
2.	Crude Protein (%)	6.60	9.46
3.	Crude Fiber (%)	4.10	5.80
4.	Crude Fat (%)	12.15	13.50
5.	Ash (%)	3.14	3.36
6.	NFE	74.01	67.88
7.	Beta Carotene	8.332mg	8.332mg

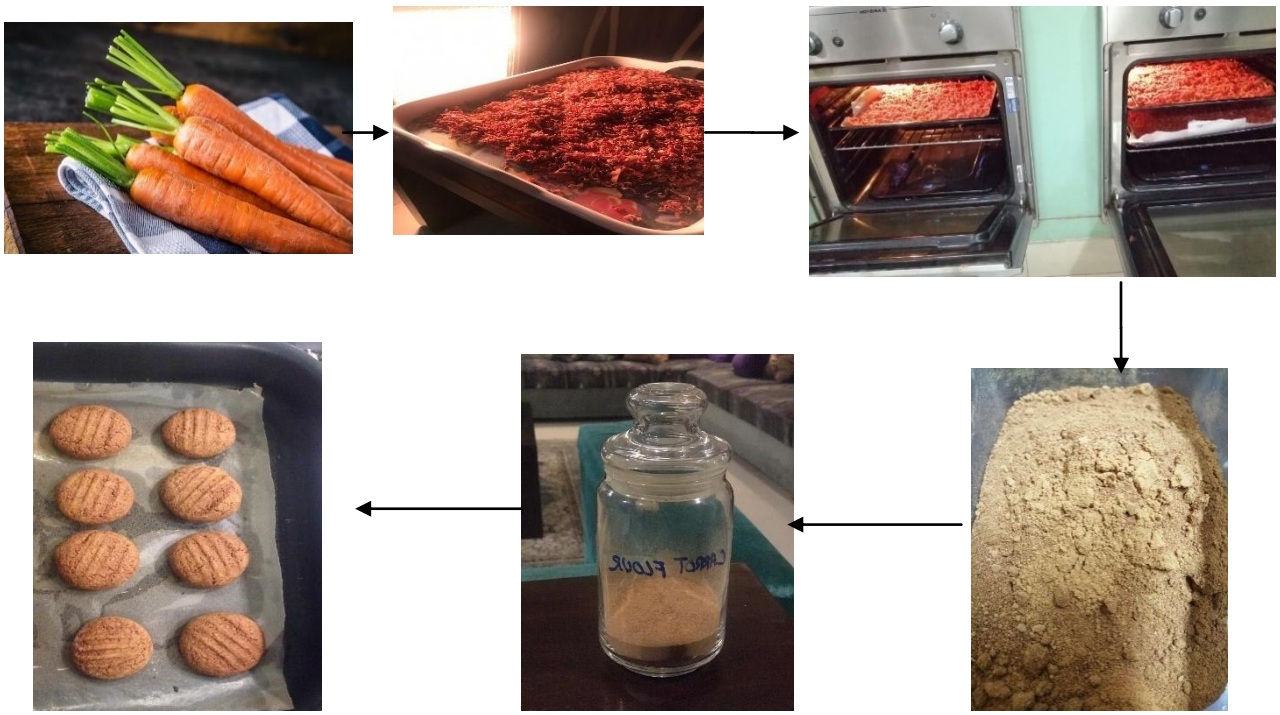

Figure 6: Development of Carrot Flour

8. **Acknowledgement**

The authors are grateful to the participants who were willing to share their experiences. We would also like to acknowledge the institute; Kinnaird College for Women, Lahore, Pakistan, for providing us with the research platform. We would also like to extend our appreciation to University of Veterinary Sciences for allowing us to conduct the proximate analysis for the products.

9. Competing Interests

The authors have declared that no competing interests exist.

10. Authors' Contributions

This work was carried out in collaboration among all authors. All authors contributed equally in various roles including formulation research goals, development of methodology, performing the experiments and analyzing data and writing the initial draft. The corresponding author coordinated the research activity as agreed by all authors. All authors read and approved the final manuscript.