

Comparative analysis of value-added cancer preventive ice-creams

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ABSTRACT

India has a rich diversity of ethnomedicinal plants that are an inseparable part of our natural wealth which plays an important role in manufacturing traditional as well as modern medicines and also act as important therapeutic agents. Ever increasing death rate in cancer patients has led to this innovative investigation using *Annona muricata*, commonly known as Graviola or Soursop possesses anti-oxidant, anti-inflammatory properties, helps in lowering blood sugar level and blood pressure, mainly serves as an anti-cancerous fruit. The variously flavoured value added Graviola ice-creams were prepared using natural preservatives, sugar substitutes without addition of stabilizing agents and artificial colours using cow's skimmed milk and plant-based source soy milk. The comparative analysis of various parameters for these ice-creams prepared using cow's skimmed milk and soy milk, beneficial for vegans and lactose in-tolerant people has been investigated. Graviola ice creams prepared using soy milk were found to have comparatively higher amounts of proteins, carbohydrates and energy. Sensory evaluation of 1000 persons for Graviola soy milk-based ice creams proved to have more overall acceptability in terms of essential parameters as compared to the Graviola ice creams prepared using skimmed milk. The Graviola ice-creams would help increase immunity, prevent liver diseases and proves to be an excellent cancer preventive as well as an enjoyable dessert by all age groups.

Keywords – anti-cancerous, anti-oxidant, anti-inflammatory, graviola, soursop, soy-milk

I. INTRODUCTION

Medicinal plants are an inseparable part of our natural wealth. India is known for its rich heritage and diversity of medicinal plants that play an important role in manufacturing a lot of traditional and modern medicines and also act as therapeutic agents. The medicinal plants possess nutraceutical properties which makes it effective for prevention or treatment in any infection and disease according to the traditional concept of Ayurveda (S, 2015). Now a days, majority of the people are affected by the dreadful disease, cancer which is the leading cause of mortality around the world. Prevention would be suitable then suffering. *Annona muricata L.*, commonly known as Graviola or Soursop possesses anti-oxidants, anti-inflammatory, anti-depressive, anti-cancerous properties as it has potential bioactive chemicals called acetogenins, that are powerful anti-tumor and anti-cancer catalysts; the consumption of the ripened fresh fruit pulp helps in improving immunity, possesses essential vitamins and large amounts of fructose, helps in lowering blood sugar level and blood pressure. It also helps rejuvenate damaged skin cells. In the present study, fresh Graviola pulp was used in preparations of ice creams by using two types of milk; cow's skimmed milk and plant-based source, soy milk which is beneficial for vegans and lactose intolerant people. Ice-creams were successfully prepared with the use of natural preservatives with sugar substitutes and use of value-added substances making it low calorie for health benefits.

II. OBJECTIVE

To prepare and develop innovative, low calorie, high nutritive value, health-based product for human benefit with the use of medicinal plant with natural preservatives.

III. MATERIALS AND METHODS

The present study was conducted to investigate value-added health based Graviola ice cream preparations and carried out the comparative analysis.

1. Preparation of Graviola ice creams

A fully ripened Graviola fruit was procured from the organic farm and the outer skin was cleaned thoroughly. De-seeded the fruit, whole fruit fresh pulp was extracted and immediately mixed with 20% fresh cream. Simultaneously, about 1-2% of corn flour and 2% condensed milk was added to 1000ml cow's skimmed milk by continuously stirring to avoid lump formation while boiling and then cooled. To this whole volume of milk about 40% freshly extracted pulp of graviola mixture was added along with mixture of 10% honey, dates and raisins respectively followed by addition of mixture of 1% natural preservative such as cinnamon and nutmeg. This 1000ml ice cream mixture was finely blended, transferred into an ice-cream container and refrigerated for about 8-10 hours. The mixture was then taken out, thawed and whisked again for about 15 to 20 minutes and was allowed to set in the freezer overnight, repeated the blending for two more times to get a smooth texture. Similarly, the graviola ice cream preparation using plant-based source soy milk was carried out. Each type of ice-creams was prepared in triplicates and further sensory evaluation was carried out.

2. Sensory evaluation of graviola ice creams preparations

Once the two types of soursop ice creams were prepared using skimmed milk (Type 1) and soy milk (Type 2) respectively, the sensory evaluation of these graviola ice creams was carried out for five characteristics and attributes such as flavour, taste, smell, texture and colour using the nine-point hedonic scale for 1000 persons and overall acceptability was calculated with their reviews.

3. Analytical testing of Graviola ice-creams

Each graviola ice-cream preparations made from plant based soy milk and skimmed milk were investigated for various parameters like fats, proteins, carbohydrates, energy, moisture and ash content/100grams. About 250grams of each type of ice-creams were analytically tested for various parameters for comparative analysis in terms of calories at Italabs Pvt. Ltd. Margao, Goa India.

4. Determination of Minimum Inhibitory Concentration (MIC) for natural preservatives (Dubey & Maheshwari, 2002)

Nutmeg, botanically known as *Myristica fragrans* (Houtt.), and Cinnamon, *Cinnamomum zeylanicum* (J. Presl.) were used as natural preservatives in the ice cream preparations of Graviola. The natural preservatives, nutmeg and cinnamon were investigated for finding its minimum inhibitory concentrations using pure cultures of *Escherichia coli* and *Bacillus subtilis* procured from the Department of Biotechnology at Goa University. The various concentrations of Nutmeg and Cinnamon was prepared using stock solutions of 1mg/ml (w/v – milligrams/milliliter) to get a wide range of working standards. Then, 0.1ml of standard test culture was inoculated in all the test tubes. Positive and negative controls were maintained for each set. All the test tubes were incubated at 37°C for 24 to 48 hours and were observed for turbidity. The lowest concentration that shows no microbial growth in the inoculated test tubes as compared with the positive and negative controls was noted.

5. Detection of the number of bacteria by SPC (Aneja, 2018)

For the determination of the number of bacteria by Standard Plate Count (SPC) method, serial dilutions up to 10⁻⁵ of the two different types of the Graviola ice-creams were prepared using saline in sterile condition and kept overnight for incubation at 37°C. About 50µl of dilution 10⁻⁴ for each type of Graviola ice-creams was plated onto the sterile nutrient agar plates and incubated at 37°C for 24 to 48 hours. The observations were noted for calculating colony forming units per millilitre (CFU/ml) using the formula as follows;

$$CFU/ml = \frac{\text{Number of colonies counted} \times \text{dilution factor}}{\text{Volume of inoculum plated}}$$

6) Microbial examination of the two types of graviola ice creams using selective enrichment media. (Aneja, 2018)

For detection of microbes in the various graviola ice cream preparations were checked by using different types of selective enrichment media. Sterile Tween-80 agar, Tomato juice agar, Milk agar, Starch agar, Glucose agar, MacConkey agar, Cetrimide agar, Eosine Methylene Blue agar, Mannitol salt agar and Czapekdox agar plates were prepared. The serial dilutions up to 10⁻⁵ of the various types of Graviola ice-creams were prepared using saline in sterile condition and kept overnight for incubation at 37°C. A loop full of the inoculum from dilution 10⁻⁴ for the two types of Graviola ice-creams was plated onto the different sterile selective enrichment media, incubated at 37°C for 24 to 48 hours and observed for selective microbial growth.

IV. RESULTS

The present study on comparative analysis of value-added cancer-preventive ice creams prepared using skimmed milk and soy milk from Graviola reveals, as evident from Table I, Fig. 1(a) that the overall acceptability of Graviola ice cream prepared using skimmed milk by sensory evaluation following the nine-point hedonic scale is 96% whereas Table II Fig. 1(b) show that the overall acceptability of the Graviola ice-creams prepared using soy milk by the sensory evaluation is 100% following the nine-point hedonic scale. As evident from Table III, the Graviola ice cream prepared using soy milk is found to be healthier in terms of low-fat content, high protein content and energy as compared to Graviola ice cream prepared using cow’s skimmed milk per 100 grams. Also, the overall acceptability in terms of flavor, taste, smell,

texture and colour of the graviola ice creams prepared using soy milk was more as compared to the graviola ice creams prepared using skimmed milk. The MIC of nutmeg used as the natural preservative (Stock - 0.5 mg/ml) was found to be 1.2 mg/ml against *Bacillus subtilis* whereas MIC of Cinnamon as natural preservative (Stock - 0.5 mg /ml) was found to be effective against *Escherichia coli* but nutmeg showed no inhibition. On comparison, Cinnamon was found to be more effective than Nutmeg as a natural preservative. Hence, a mixture of nutmeg and cinnamon was used as a natural preservative in both types of Graviola ice cream preparations. Selective enrichment media used to check the growth of various microbes on different media for Graviola ice-cream preparations showed absence of proteolytic bacteria, *Staphylococcus aureus*, *Lactobacilli*, *Bacillus cereus*, *Pseudomonas sp.* and *Enterobacteriaceae* as seen in Table IV. But, both types of Graviola ice-creams prepared using skimmed milk as well as soy milk indicated presence of *E. coli* in permissible limits.

I. Sensory evaluation of Graviola ice creams using skimmed milk

Grading categories		Charcateristics and attributes					Overall acceptability
		Flavour	Taste	Smell	Texture	Colour	
Like	Extremely	56	64	16	04	04	28.8%
	Very much	36	36	36	28	56	38.4%
	Moderately	08	-	28	40	24	20%
	Slightly	-	-	20	16	08	8.8%
Neither like nor dislike		-	-	-	-	-	-
Dislike	Slightly	-	-	-	12	-	2.4%
	Moderately	-	-	-	-	-	-
	Very much	-	-	-	-	08	1.6%
	Extremely	-	-	-	-	-	-

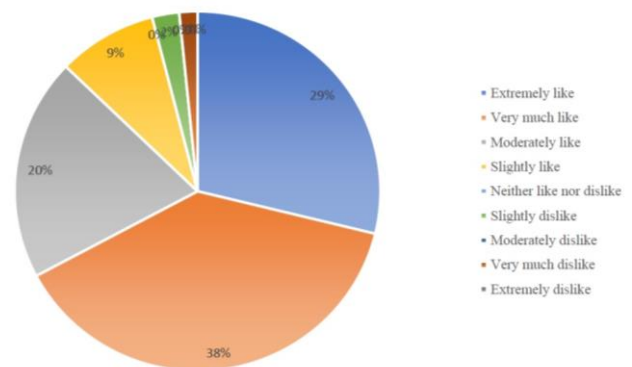


Fig. 1(a) Sensory evaluation of 1000 person for Graviola ice creams prepared using skimmed milk using 9-point hedonic scale.

II. Sensory evaluation of Graviola ice creams using soy milk

Grading categories		Charcateristics and attributes					Overall acceptability
		Flavour	Taste	Smell	Texture	Colour	
Like	Extremely	44	44	32	32	28	36%
	Very much	28	24	24	40	36	30.4%
	Moderately	28	28	20	16	28	24%
	Slightly	-	04	24	12	08	9.6%
Neither like nor dislike		-	-	-	-	-	-
Dislike	Slightly	-	-	-	-	-	-
	Moderately	-	-	-	-	-	-
	Very much	-	-	-	-	-	-
	Extremely	-	-	-	-	-	-

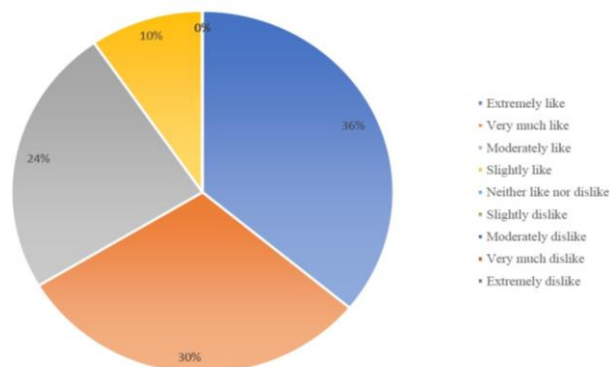


Fig. 1(b) Sensory evaluation of 1000 person for Graviola ice creams prepared using soy milk using 9-point hedonic scale.

III. Comparative analytical report of various parameters

Sr. no.	Various Parameters	Analytical results (per 100gm)	
		Type 1 Graviola ice cream	Type 2 Graviola ice cream
1.	Fats	3.90 gm	3.61 gm
2.	Proteins	2.65gm	4.03 gm
3.	Carbohydrates	17.77gm	23.22 gm
4.	Energy	116.78 Kcal	141.49 Kcal
5.	Moisture	74.87%	68.25%
6.	Ash	0.81%	0.89%

V. Microbial growth checked using selective enrichment media

Sr. no.	Types of selective enrichment media	Graviola ice-cream (skimmed milk)	Graviola ice-cream (soy milk)
1.	Cetrimide agar	-ve	-ve
2.	Czapekdox agar	-ve	-ve
3.	EMB agar	-ve	-ve
4.	Glucose agar	-ve	-ve
5.	MacConkey agar	+ve	+ve
6.	Milk agar	-ve	-ve
7.	Mannitol salt agar	-ve	-ve
8.	Starch agar	-ve	-ve
9.	Tomato juice agar	-ve	-ve
10.	Tween-80 agar	-ve	-ve

*Note: '+ve' indicates presence and '-ve' indicates absence.

V. CONCLUSION

The former studies of *Annona muricata* L., Graviola shows promising anti-cancerous activity, hence in the present study and investigation, its use in preparation of ice-cream would impart its properties and provide prevention against abnormal cell growth as it proves to be a preventive measure and an enjoyable dessert that can be consumed by all age groups Ice-creams prepared using plant soy milk was found to be healthier than that prepared using cow's skimmed milk as it was found to be low calorie and rich in protein content and energy having 100% overall acceptability in terms of flavor, taste, smell, texture and colour as well as good benefits for lactose in tolerant as well as health conscious people hence, it hold manifold profits to start-ups.

VI. FUTURE SCOPE

Future work on this area of study could focus on testing and extending the shelf life. Natural colours can be used for making the ice-cream look more presentable and attractive to

the costumers in case of commercialization. The soursop ice-cream can be formulated using a shot of liquid nitrogen which would give it a smooth texture preventing formation of

ice crystals. Insights from this research can help in encouraging the emerging entrepreneurs in formulating the market strategies.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- [1] Badrie, Neela & Schauss, Alexander. (2010). Soursop (*Annona muricata* L.): Composition, nutritional value, medicinal uses, and toxicology. 10.1016/B978-0-12-374628-3.00039-6.
- [2] Khurshid A., Jawed A. Siddiqui. (2018). Emerging therapeutic potential of graviola and its constituents in cancers. *Carcinogenesis*, Volume 39, Issue 4, 5 April 2018, Pages 522–533,
- [3] Ana V. Coria-Télez Efigenia Montalvo-González Eva N. Obledo-Vázquez. (2017). Soursop (*Annona muricata*). John Wiley & Sons, Ltd. February 2017.
- [4] Diana Escobedo-López, et al., Priority areas to collect the germplasm of *Annona* (*Annonaceae*) in Mexico based on diversity and species richness indices, *Genetic Resources and Crop Evolution*, 10.1007/s10722-018-0718-2, (2018).
- [5] Gagan D., Rahul K., (2016). Graviola inhibits hypoxia-induced NADPH oxidase activity in prostate cancer cells reducing their proliferation and clonogenicity. *Sci Rep.* 2016; 6: 23135. PMID: PMC4793251. PMID: 26979487.
- [6] Soheil Zorofchian M., Sonia Nikzad, et al., *Annona muricata* (*Annonaceae*): A Review of Its Traditional Uses, Isolated Acetogenins and Biological Activities. *Int J Mol Sci.* 2015 Jul; 16(7): 15625–15658. PMID: PMC4519917.
- [7] Gupta, R. (April 2015). A Study of Anti Diarrhoeal effects of Nutmeg. *INT.J. Pharma. SCI. REV. RES.*, 94-96.
- [8] Gyurova, D. K. (2014). Dried Fruits-Brief Characteristics of their Nutritional Values *Journal of Food and Nutrition Science*, 105.
- [9] Patrikios Ioannis, S. A. (Jan 2016). Graviola- A systematic Review on its Anti-cancer properties. *American Journal of Cancer Prevention*, 128-129.
- [10] Patil, K. (Nov 2018). 13 Proven health benefits of Dates. *Organic Facts*, 1.
- [11] Rajendra Gupta*, J. L. (2015). A Study of Antidiarrhoeal Effects of Nutmeg. *Int. J. Pharm. Sci. Rev. Res.*, 31(1), March – April 2015, 1-2.
- [12] Peryam, D.R. and Pilgrim, F.J. 1957. Hedonic scale method of measuring food preferences. *Food Technology* (September 1957), 9-14
- [13] Lim, Juyun. (2011). Hedonic scaling: A review of methods and theory. *Food Quality and Preference - FOOD QUAL PREFERENCE*. 22.733-747. 10.1016/j.foodqual.2011.05.008.
- [14] <https://www.sensorysociety.org/knowledge/sspwiki/pages/the%209-point%20hedonic%20scale.aspx> (Online as on 23/08/18 at 15:34).
- [15] Juyun Lim. Hedonic scaling: A review of methods and theory. *Elsevier Food Quality and Preference* 22 (2011) 733–747.