

DEVELOPMENT OF WHEATGRASS AND CARROT BLENDED JUICE

व्हीटग्रास एवं गाजर मिश्रित जूस का विकास

Thesis

Master of Technology in Agricultural Engineering

(Processing and Food Engineering)

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Department of Processing and Food Engineering

College of Technology and Engineering

Maharana Pratap University of Agriculture & Technology, Udaipur

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**Master of Technology in Agricultural Engineering
(Processing and Food Engineering)**



By

Vivek Kumar

2023

**COLLEGE OF TECHNOLOGY AND ENGINEERING
MAHARANA PRATAP UNIVERSITY OF AGRICULTURE AND
TECHNOLOGY, UDAIPUR**

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CERTIFICATE – III

This is to certify that this thesis entitled “**Development of Wheatgrass and Carrot Blended Juice**” submitted by **Mr. Vivek Kumar** to Maharana Pratap University of Agriculture & Technology, Udaipur, in partial fulfillment of the requirements for the degree of **Master of Technology** in Agricultural Engineering in the subject of **Processing and Food Engineering** was after recommendation by the external examiner and defended by the candidate before the following members of the examination committee. The performance of the candidate in the oral examination on his thesis has been found satisfactory; we therefore, recommend that the thesis to be approved.

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This is to certify that **Mr. Vivek Kumar** student of Master of Technology in Agricultural Engineering in the subject of Processing and Food Engineering, Department of Processing and Food Engineering has made all corrections/modifications in the thesis entitled **“Development of Wheatgrass and Carrot Blended Juice”** which were suggested by the external examiner and the advisory committee in the oral examination held on 17/10/2023. The final copies of the thesis duly bound and corrected were submitted on / / 2023 are enclosed here with approval

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Per cent
°C	Degree Celsius
ANOVA	Analysis of variance
AOAC	Association of Analytical chemists
CTAE	College of Technology and Engineering
wb	Wet basis
Eqn.	Equation
<i>et al.</i>	et alibi, and others
etc.	et cetera
Fig.	Figure
g	Gram
db	Dry basis
kg	Kilo gram
WJ	Wheatgrass juice
CJ	Carrot juice

CHAPTER I

INTRODUCTION

India with diverse agro climate ensures the availability of all varieties of fruits and vegetables. Fruits and vegetables supply a variety of micronutrients, including minerals, vitamins and antioxidant components like carotenoids and polyphenols, in addition to the main dietary fibre component of food (Augspole *et al.*, 2014). India is second in the world after China in terms of fruits and vegetables production. India contributes 13 per cent and 10.7 per cent, respectively, to the global production of fruits and vegetables (National Horticulture Database, 2021-22). In 2021-22; the total production of horticultural crops accounts 341.63 million metric tons out of which production of fruits and vegetables are 107.10 Million metric tons and 204.61 million metric tons respectively. Per capita availability of vegetables in India is 401 g per day but recommended quantity of fruits is 300 g per capita per day (National Horticulture Database, 2021-22).

The *Umbelliferae* family includes the vital root vegetable known as the carrot, which is grown all over the world. It is sown between August and November, and after 75 to 80 days of maturation, it is harvested. The body benefits substantially from phyto-nutrients such bioactive compounds, carotenoids, minerals, and vitamins, which are abundant in carrots and their products, including juices (Jabbar *et al.*, 2014). The common orange carrot is a good source of vitamin A and is also contain α - and β -carotene. The two main antioxidant pigments in carrots are carotenoids and anthocyanins. Carrots are orange in colour because of the antioxidant β -carotene. During digestion, β -carotene is absorbed in the intestines and transformed into vitamin A (Singh *et al.*, 2021). Carrot is consumed both in the raw and cooked forms. This is used to make pickles, juice and halwa. Carrots may be effectively preserved for the off-season using techniques including dehydration, canning and pickling. One of the crucial ways to add value to food material and make them available in the off season is by dehydration. Even abroad, there is a market for dehydrated carrots and its juice (Kukanoor *et al.*, 2014).

Carrot is grown throughout the world. China ranks first in the carrot production in the world. In India, total growing area of carrot 110 hectares with production of 1910 tons in 2021-22. Rajasthan produce 15.51 tons of carrot in a 3.99 hectares of land (National Horticulture Database, 2021-22).

The carrot has moisture content 86–90 %, and the rest of the composition being made up of 7-10 % carbohydrates, 3% fiber, 1% protein, 1% ash and 0.2% fat. Simple sugars and cellulose, respectively, make up the majority of the fiber and carbohydrate contents. Thiamin, riboflavin, and niacin are all present in sufficient proportions in carrot (Nicolle *et al.*, 2004). The high β -carotene concentration in carrots gives them orange colour, is another reason for their popularity (Arscott and Tanumihardjo, 2010).

The word wheatgrass often refers to the sprouts produced by the germination of wheat seeds during a period of 6 to 10 days (Akbas *et al.*, 2017). Grasses are recognized to provide regenerative and health-protective characteristics for animals and humans, all over the world. Wheatgrass is an excellent source of calcium, iron, sodium, potassium and magnesium. It is also a good source of trace minerals, B vitamins, vitamin A, vitamin E and vitamin K. Wheatgrass also contains eight active enzymes, seventeen amino acids, and chlorophyll (Pallavi and Shikha, 2016). Wheatgrass juice is liquid extract from mature sprouts of wheat seeds (Rodriguez *et al.*, 2022). Due to rising knowledge of wheatgrass juice's nutritional benefits and health-improving properties over the past 20 years, its popularity has increased. Antioxidant-rich wheatgrass is now more well recognized and is accessible in number of forms, including beverages and pills. Foods high in antioxidants offer several benefits, including the ability to slow down the ageing process and guard against chronic illnesses like cancer and Alzheimer's (Nanasombat *et al.*, 2015).

Wheatgrass juice is one of the major sources of antioxidants with plenty of health advantages and chlorophyll, which makes up to 70% of the total chemical components (Ghumman *et al.*, 2017). Chlorophyll is well-known for its abilities to prevent cancer, as well as to protect against the side effects of cancer treatment. Chlorophyll was shown to be the active component in wheat sprout root and leaf extracts, which were proven to reduce carcinogen metabolic activity (Jain *et al.*, 2014). Wheatgrass contains phenolic compounds that counter the effects of oxidative stress and lower the rate of a number of degenerative illnesses and disorders (Calzuola *et al.*, 2004).

Justification

Functional beverages provide a number of benefits. Consuming nutritious food and beverages on a daily basis is essential in preventing disease. Consuming food or beverages with functional qualities can be an alternative to leading a healthy lifestyle since they include bioactive components that can fight free radicals. The healthy food and beverages must not only be rich in nutrients but also free from physical, chemical and microbiological contamination. Wheatgrass and carrots are both powerhouses of essential nutrients. Wheatgrass is abundant in vitamins A, C, E and K, as well as minerals like iron, magnesium and calcium. On the other hand, carrots are known for being rich in beta-carotene vitamin K, vitamin-C and potassium (Pamela *et al.*, 2021).

Population growth throughout the world have decline in the nutritional content in their die. As a result, individuals will increasingly need to turn to alternate food sources or dietary supplements to get the rapidly absorbable vitamins, minerals, chlorophyll and enzymes, for stronger immune systems (Pallavi and Shikha, 2016). With significant levels of vitamins, minerals, amino acids, active enzymes, antioxidants and chlorophylls, wheatgrass juice provides a rich source of nutrition (Kaur *et al.*, 2021). Additionally, it is gluten-free and good for allergic customers (Qamar *et al.*, 2019). The juice includes carotenes in the range of 59.4–94.5 mg/100ml juice and vitamin-C in the range of (30.8 mg/100ml juice) Both wheatgrass and carrots contain antioxidants, which helps to combat oxidative stress and reduce damage caused by free radicals in our body. Regular consumption of antioxidant-rich foods can contribute to reducing the risk of chronic diseases and support a healthy immune system (Skoczylas *et al.*, 2018).

Carrots are high in dietary fiber. When eaten often, carrots act as a barrier of defence for our bodies. Like many other vegetables, carrots also have a lot of antioxidants. The biological and therapeutic advantages of carrots may result from their high content of antioxidant carotenoids, especially β -carotene. Carotenoids, polyphenols, and vitamins are same as of antioxidants found in carrots. Orange carrots are rich in carotenoids, which are strong antioxidants that may mitigate the effects of free radicals. Flavonoids and phenolic derivative are both antioxidants found in carrot roots (Varshney *et al.*, 2022). This research work was carried out to develop juice by blending wheatgrass and carrot juice due to its associated health

benefits and therefore present research work was carried out with following specific objectives:

1. To develop the juice by blending of wheatgrass and carrot
2. To evaluate the quality of developed juice
3. To study the storage life of developed juice

CHAPTER-II

REVIEW OF LITERATURE

The literature pertaining to different aspects of the present study has been reviewed and enlisted below:

- 2.1 Wheat production data
- 2.2 Carrot production data
- 2.3 Nutritional status of wheatgrass and carrot
- 2.4 Blending of juice
- 2.5 Wheatgrass and carrot product
- 2.6 Pasteurization of juice
- 2.7 Storage

2.1 Wheat Production Data

Sharma *et al.*, (2015) reported wheat as the major crop which fulfills the energy requirement of human diet all over the world. Due to the availability of a wide range of end products at cheaper prices than other cereal crops, the demand for wheat has expanded. From its present output level of wheat, the FAO estimates that the world will need about 940 million tons of wheat by 2050. To fulfill this need, developing nations must boost wheat output by 77%.

Additionally there is a need to increase wheat production through agronomic (water, nutrients and weed control *etc.*), genetic, physiological interventions and also resource conservation technologies. To meet the future demands, basic and strategic research on crop modeling for advance forecast yields, adaptation and monitoring of climate change will be helpful.

The total production of wheat in the world is around 764.981 million tons with the production area of 215.900 million ha. India ranks second in the world with an annual production of 109.596 million tons having production area of 31.61 million ha with Uttar Pradesh, Madhya Pradesh, Punjab, Haryana, Rajasthan, Bihar, Gujarat and Maharashtra being the major producing states (Table 2.1) (Anonymous,2022).

Table 2.1 Major producing states of wheat (2021-22)

State	Area(HA)	% of all India	Production(MT)	% of all India
Uttar Pradesh	9.85	31.16	35.50	32.42
Madhya Pradesh	6.39	20.20	17.62	16.08
Punjab	5.23	11.15	17.14	15.65
Haryana	2.56	8.08	12.36	11.28
Rajasthan	3.00	9.50	11.04	10.08
Bihar	2.22	7.02	6.34	5.79
Gujarat	1.02	3.22	3.26	2.98
Maharashtra	1.31	4.13	2.33	2.13
Other	1.75	5.54	3.94	3.60
Total	31.61	100.00	109.52	100.00

(Source: Agricultural Statistics at a glance, 2022)

2.2 Carrot Production Data

Sun *et al.*, (2021) mentioned horticultural crops as an essential component of agriculture for food and nutritional requirement. In recent years, horticulture crops have drawn a lot of interest due to improvements in their growth, productivity and quality. Horticulture significantly improved land productivity, created jobs, enhanced exports, improved the financial situation of farmers and business owners, and also ensured the public's access to food and nutrition.

The total production of carrots in India is around 1910.24 metric ton with the production area of 110.22 ha. Haryana ranks first in India with an annual production of 386.39 ton having production area of 31.61 ha with Uttar Pradesh, Madhya Pradesh, Punjab, Rajasthan, Bihar, Tamil Nadu, Karnataka, Assam, West bengal being the major producing states (Table 2.2) (Anonymous, 2022).

Table 2.2 Major producing states of carrot (2021-22)

States	Area(HA)	Production(MT)
Haryana	22.400	386.390
West Bengal	13.18	235.39
Punjab	9.24	224.74
Uttar Pradesh	7.05	178.97
Madhya Pradesh	8.84	167.59
Bihar	13.92	147.53
Tamil Nadu	5.77	140.52
Karnataka	5.03	96.63
Assam	4.66	77.53
Telangana	2.58	44.28
Jammu & Kashmir	1.88	32.32
Chhattisgarh	2.33	30.60
Meghalaya	1.28	23.89
Maharashtra	1.98	19.61
Andhra Pradesh	0.82	17.24
Rajasthan	3.99	15.51
Others	5.27	71.5
Total	110.22	1910.24

(Source: Agricultural Statistics at a glance 2022)

2.3 Nutritional Benefits of Wheatgrass and Carrot

Sharma (2008) revealed that adding wheatgrass juice to a diet considerably raised hemoglobin levels, going from 9.35% to 12.98% in 30 days. Following supplementation, the amount of glutathione in whole blood increased to 76.18% from 42.78%. When used as a food supplement in the treatment of various problems, wheatgrass juice has real therapeutic benefits for the body.

Mujoriya *et al.*, (2011) reported that human body benefits greatly from the minerals and vitamins found in wheatgrass. The chemical composition of wheatgrass juice is very comparable to that of blood. Both have same pH (7.4) and have fast absorption. Being an excellent source of calcium, wheatgrass supports the health of

bones and teeth and also helpful in regulating heart rate and acting as a buffer to bring blood pH levels back into equilibrium.

Sharma *et al.*, (2012) reported that carrots as one of the key root vegetables having vitamin, minerals, carotenoids and dietary fibres, as well as notable amounts of a number of other functional elements that have vital health-promoting effects (Table 2.3). Due to its significant source of naturally occurring antioxidants with anticancer potential, the consumption of carrots and the products derived from them is constantly rising.

Table 2.3 Nutritional profile of carrot juice

Vitamin and minerals	Amount (mg/100ml)
Zinc	88
Potassium	45
Magnesium	42
Phosphorus	15
Calcium	0.7
Sodium	0.4
Iron	0.38
Folate	0.31
Vitamin K	24
Vitamin A	3.60
Vitamin C	0.2

Singh *et al.*, (2021)

Judita *et al.*, (2015) reported that β -carotene and vitamins (A, B and C) are abundant in carrots. Additionally, it includes pantothenic acid, folic acid, calcium and magnesium. Carrot consumption enhances digestion, decreases cholesterol and improves vision. Five types of carrot varieties (Jitka, Kardila, Katlen, Rubna and Koloseum) samples were examined for total polyphenols, β -carotene, and antioxidant activity. The total amount of polyphenols in the samples varied from 81.25 mg/kg to 113.69 mg/kg, while the amount of β -carotenes in the samples ranged from 24.5

mg/kg to 124.28 mg/kg. Selected carrot types have antioxidant activity ranging from $6.88 \pm 0.92\%$ to $9.83 \pm 0.62\%$. The carrot variety of koloseum was found to contain the maximum amount of total polyphenols (113.69 ± 11.57 mg/kg), which was statistically significant. Additionally, koloseum has the highest antioxidant activity ($9.83 \pm 0.62\%$) and the highest β -carotene concentration (124.28 ± 3.54 mg/kg).

Chouhan (2014) determined the nutritional profile of wheatgrass juice (Table 2.4). Further they mentioned that concentration of elements such as ascorbic acid, dehydroascorbic acid, carotene, potassium, phosphorus, calcium, sulfur, sodium, aluminum and copper increased linearly in the shoots with the growth period, whereas the concentrations of the elements, namely zinc, magnesium and iron, remained constant in the shoots after the eighth day of plant growth.

Table 2.4: Nutritional profile of wheatgrass juice

Vitamin & minerals	Amount(mg/100ml)
Potassium	57
Ascorbic acid	25.2
Phosphorus	8.2
dehydroascorbic acid	7.6
Carotene	2.43
Calcium	2.4
Magnesium	1.7

Thakur *et al.*, (2019) mentioned wheatgrass is herb and could save lives owing to its nutritional and medicinal characteristics. Wheatgrass has a high concentration of chlorophyll, which enhances its ability to scavenge free radicals. Its juice contains vitamins, macronutrients and other nutrients that accelerate anti-oxidative processes and decrease the impact of degenerative disorders including cancer, artery disease, diabetes, thalassemia and atopic dermatitis.

Eissa *et al.*, (2020) developed high quality; nutritious, ready-to-drink juice with a suitable amount of vitamin-C using wheatgrass juices to improve lifestyle. Its beverage has high levels of the vitamins B₁ (thiamine), B₃ (nicotinic acid) and B₁₂ available. Due to its high concentration of bioactive compounds, it may be suggested to drink it as a juice blend to increase fertility and look young. The current consumer's shifting lifestyle, has considerably changed the marketing trends in the food business, could be is taken into account for increasing the acceptability of this juice. This juice is strongly advises because of their importance for both micronutrients, macronutrients and, as well as their positive effects on health.

2.4 Blending of Juice

Bhardwaj and Mukherjee (2011) blended kinnow juice: aonla juice: ginger juice in ratios of 100:0:0, 95:5:0 and 92:5:3 and kinnow juice: pomegranate juice: ginger juice in ratios of 90:10:0 and 87:10:3 to improve flavor, palatability and nutraceutical properties. Pomegranate juice carried small per cent of variation in TSS (12.00 to 14.13°Brix), acidity (0.720 to 0.510%), ascorbic acid (18.38 to 12.90 ml/100 ml juice) and limonin (0.103 to 0.250 ml/100 ml juice). The best juice mixture was kinnow juice: pomegranate juice: ginger juice (87:10:3). The sensory evaluation score was higher and the consistency and flavor ratings were improved till the end of storage. The juice blend ratio of kinnow, aonla and ginger (92:5:3) exhibited the least amount of non-enzymatic browning (0.081 to 0.104ml/100ml juice) and the lowest populations of bacteria (4.0×10^3), mould (1.5×10^3), and yeast (2.1×10^3) at the end of storage (six months).

Eriq, (2011) reported that blended juice from 70% carrot juice and 30% orange juice (V/V) have a high acidity pH of 3.54. There were 94.33 percent of water, 6.0°Brix of soluble solids and 7.55 ml/100ml of total carotene, respectively. The juice had low levels of B vitamins and protein but high levels of ascorbic acid (20.73mg/100 ml). The juice contains 10.5 mg/100ml of total sugar and 76.83 mg/ml of reducing sugar, respectively.

Ishak *et al.*, (2018) evaluated the properties and preventive benefits of pomegranate juice (PJ), lemon juice (LJ), wheatgrass juice (WGJ) and their functional properties. The research revealed that the wheatgrass juice (WGJ) have low acidity.

While pomegranate juice (PJ) and lemon juice (LJ) have noticeable acidity, the mixed juice has more acidity overall. With a significant 98.16% ability to scavenge free radicals, the results showed that functional blended juice had the best antioxidant activity. The blend of wheatgrass juice (WGJ): pomegranate juice (PJ): lemon juice (LJ) 20:70:10 was found to be the most preferred by sensory analysis.

Vignesh *et al.*, (2019) conducted a study to evaluate the sensory and storage properties of the RTS juice enhanced with papaya leaves flavonoids. The sample of papaya, carrot and mango fruits was accepted for the sensory evaluation with a mix ratio of 60:10:30. The presence of flavonoids has been found to be helpful for increasing the product's shelf life as well as maintaining the nutritional properties of the final RTS product.

Vichaibun and Kanchanaphu (2019) studied the impact of lemon on the chemical and functional properties of different juices. The overall phenolic content of the blends was raised by additional lemon juice, especially in the blends of pineapple juice. The 50:50 (v/v) mixtures of pineapple juice and lemon juice had the maximum phenols. As the amount of lemon juice increased, the total antioxidant content of the juice combinations significantly increased as compared to 100% pure fruit juice. The study revealed the health advantages from varying lemon juice per cent in the juice blends raised their total phenolic content of 42 mg/100ml even if the blends overall antioxidant capacity had no synergistic effect.

2.5 Wheatgrass and Carrot Products

Das *et al.*, (2014) reported that mixing of wheatgrass powder into the idli batter increased the amount of phenolic compounds but lowered the volume of the product. The product hardness was related to batter volume. Wheatgrass powder added into batter up to 1%, as per the sensory panel. The results shows wheatgrass enriched idli with a concentration of 1-2 per cent had higher levels of B vitamins and vitamin C.

Nanasombat *et al.*, (2015) reported that wheatgrass is currently offered in a number of forms, including beverages and capsules and has gained recognition as it is a rich source of antioxidants. Foods that contain antioxidants provide several benefits,

including the ability to slow down the ageing process and guard against chronic illnesses like cancer and Alzheimer's.

Rahman *et al.*, (2015) used wheatgrass powder in place of wheat flour in muffin recipes at varying per cent of 0, 2.5, 5.0 and 7.5. The viscosity, hardness, chewiness, protein, total dietary fiber and total phenolic content of the muffin batter all increased with increase in wheatgrass powder. On the other hand, sample volume, cohesiveness, springiness and colour showed the opposite tendency. The sensory acceptability of muffins with 7.5 per cent wheatgrass powder was lower when compared to other levels, whereas 5.0 per cent received the highest score (8.4).

Sultana *et al.*, (2016) used carrot, green chilli, and brinjal as the main ingredients in the mixed fermented vegetable pickles. A 10% salt solution, 8% salt + 1% sugar solution and 8% salt + 1% sugar + 1% acetic acid solution shows that acidity attained maximum value for the samples which were kept in 8% salt + 1% sugar + 1% acetic acid. High concentration of salt (10% salt) results in low acid production. Total viable bacteria were also less in the pickle which was fermented in 8 % salt + 1 % sugar and 1 % acetic acid and followed by the pickle which was fermented in 8% + 1% sugar, 10% salt solution respectively. Throughout the four months of storage, no fungus growth could be seen. Organoleptic taste revealed that the panelists approved all pickles samples, rating all fermented pickles as "like moderately" while rating fresh pickles as "like slightly". The samples that were fermented in solutions of 10% salt, 8% salt + 1% sugar received the highest average score. Fermented pickles did not change in colour, flavor or texture after storage at room temperature (22°C–28°C) for the first four months, although unfermented pickles did alter in flavor and texture slightly beyond that time.

Vairagade *et al.*, (2016) prepared carrot *halwa* by substituting sugar in recipe with saccharine at four different amounts. The study found that saccharine as a low calorie sweetener may be replaced up to 10% sugar used in preparing carrot halwa. This could lower calorie content of the food while preserving its sweetness and sensory qualities at par with original product (control) till 5 days when stored at 10°C. The standard plate count, yeast and mould microbiological counts were within acceptable limits.

Nayi *et al.*, (2023) prepared porridge by using carrot pomace (10–50 g/100 g) and sweet corn by adding milk (50–80 g/100 g) and small amount of sugar (12–20 g/100 g). The colour value in terms of L* and b* of developed porridge increased with the increase in the amount of milk while the color a* value decreased with an increase in the amount of milk. The pH value decreased with an increase in the amount of milk, whereas the TSS value decreased with an increase in cooking time and sugar content, while ascorbic acid also decreased because of the interaction of sugar and cooking time. Reducing sugar was found to increase with the increase in sugar content, while total sugar content was increased with the increase in added sugar. Overall acceptability of porridge was found to increase with the increase in carrot pomace (7.82) was finalized at 35 g carrot pomace, 100 ml milk, 16.00 g sugar and 13 min cooking time.

2.6 Pasteurization of Juice.

Hirdyani (2015) evaluated quality of RTS beverages made from kinnow juice, basil extract, ginger and sugar syrup by pasteurizing at 90°C for 25s, cooled and stored at 5°C refrigerated temperature for 20 days. Marginal changes in pH, total soluble solids, acidity, vitamin C and antioxidant content were observed. The TSS was increased by 2.5°Brix. Addition of basil extract and ginger to kinnow juice increased the antioxidant potential. The Vitamin C, pH and subsequently acidity decreased with duration of storage. The mean overall acceptability score was more than eight for beverage samples up to 20 per cent basil extract incorporation.

Chakraborty and Bhagat (2022) treated pomegranate juice with pulsed light (PL) in the range of 312-2988 J/cm² to 761.4 J/cm² (2.7 kV for 90s), a 5-log reductions in (microbial safety) in the count of *Escherichia coli* ATCC 43888, aerobic mesophiles, yeasts, and moulds was achieved in the juice. The corresponding lethality was reached with a heat treatment lasting two minutes at 95°C. The treatments at 2988 J/cm² pulsed light (PL) and 95°C/3 min (thermal treatment) completely inactivated the enzyme activity (polyphenol, oxidase and peroxidase) and ensured microbiological safety. Only 34% vitamin C, 30% phenolic components and 37% antioxidant capability was present in the thermally pasteurised sample (95°C/3 min). The phenolics, antioxidants and ascorbic acid 97%, 94% and 83% respectively, were present in the pulsed light (PL) pasteurized (2988 J/cm²) juice. Pulsed light (PL)

treatment of the pomegranate juice at 2988 J/cm² meets the requirements of enzyme stability, phytochemical retention and microbiological safety.

2.7 Storage

Dhaliwal and Hira (2001) prepared four distinct combinations of carrot juice and stored for six months in glass bottles at room temperature (25°C) with blending or two levels each of beetroot (5 and 10 %) and black carrot (10 and 20 %) juice. While storage for 6 months led to (71.26-80.28 %) losses in ascorbic acid and (56.60-66.57 %) losses in β -carotene content, pasteurisation caused losses of (11.11-13.09 %) ascorbic acid and (14.00-20.47 %) β -carotene.

Calskantur *et al.*, (2011) reported that although only slight variations in antioxidant activity and total polyphenols, it was noticed that the vitamin C content of juice samples considerably reduced with increasing time and temperature. The amount of vitamin C in whole orange juice dropped by 22, 30 and 47%, respectively, after 4 weeks of storage at 6, 15 and 30°C.

Attri *et al.*, (2012) tested physico-chemical and sensory characteristics of malta-ginger squash found and it was reported that the squash made from malta (20%) and ginger (5%) found best in sensory assessment both at the time of preparation and after 3 and 6 months of storage. While the total soluble solids and total sugars substantially increased over the six-month storage period, it was discovered that the acidity, ascorbic acid, reducing sugars and antioxidants reduced.

Satkar *et al.*, (2013) prepared RTS beverage of bitter gourd by balancing the amounts of fruit juice, sugar and citric acid. The typical ingredients for fresh RTS were 15 ml of juice, 15 g of sugar, 0.29 g of citric acid and 76 ml of water. Even after three months of storage, the RTS kept an under refrigeration temperature was found to be acceptable.

Bolaji and Akanbi (2017) assessed the antioxidant properties and storage stability of the improved aloe vera, lemon and orange combination. Aloe vera gel in range of 40 to 70 %, lemon juice of 20 to 40% and orange juice of 30 to 60% were combined to create a healthful ready-to-serve beverage. Aloe vera gel, lemon juice and orange juice made up the blend actual optimised composition at the ratios of 47:14:39, respectively. The pH, total soluble solids, total titratable acidity and sensory ratings of the juice mix did not change significantly during storage, however after 60 days of

storage, there had been a 66% and 35% loss of vitamin C and total phenol content, respectively. The aloe vera, lemon and orange mixture displayed pleasing sensory qualities and was stable in storage.

CHAPTER III

MATERIALS AND METHODS

This chapter describes the experimental setup, procedures and approaches used to accomplish the current research on “Development of Wheatgrass and Carrot Blended Juice” carried out in the Department of Processing and Food Engineering, Bio-Processing Lab of All India Co-ordinated Research Project on Post-Harvest Engineering and Technology, College of Technology and Engineering, Dairy-Chemistry Lab and Dairy and Food Engineering Lab of College of Dairy and Food Technology, Department of Horticulture, Rajasthan College of Agriculture at Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan. The following headings provide descriptions of the materials and procedures used:

3.1 Collection of raw material

3.2 Growing of wheatgrass

3.3 Cleaning and sorting

3.4 Extraction of juice

3.5 Preparation of wheatgrass and carrot based blend juice

3.6 Physico-chemical analysis of developed and stored product

3.7 Microbiological analysis

3.8 Sensory evaluation of fresh juice

3.9 Statistical analysis

3.1 Collection of Raw Materials

Wheat (variety Raj 4238) seeds (Plate 3.1) were procured from the nearby local market of Udaipur. It was grown and cut on eighth days after the sowing. Fresh carrots (Plate 3.2) of similar ripeness, having uniform visual quality, maturity and sizes were collected.



Plate 3.1: Wheat seeds



Plate 3.2: Raw Carrots

3.2 Growing of Wheatgrass

Wheat seeds were collected from the market as mentioned earlier. Seeds of wheat were soaked in water for 12 hours. After soaking (Plate 3.3) wheat seeds were wrapped in a cotton cloth and kept wet for the remaining period. Sprouting started after 12 hour. Soil was prepared (Plate 3.5) before sowing. The sprouted seeds (Plate 3.4) were uniformly spreaded (Plate 3.6). The seeds were placed near to one another and then they were covered with a thin layer of soil (about 2-3 cm thick) and then water was sprinkled.



Plate 3.3: Wheat Seed soaked in water Plate 3.4: Germinated wheat seed



Plate 3.5: Soil bed preparation

Plate 3.6: Sowing of seed



Plate 3.7: Wheatgrass

Plate 3.8: Cutting of wheatgrass

3.3 Cleaning and Sorting

Fresh carrots were brought from local market of Udaipur. Damaged and defective carrots were removed. Dust and contaminants were removed with clean water.

Wheatgrass (Plate 3.7) roots were cut and that were washed with water to remove soil and other unwanted material adhered to the samples (Plate 3.10).



Plate 3.9: Cleaned and sorted carrot



Plate 3.10: Cleaned and sorted wheatgrass

3.4 Extraction of Juice

After 8 days, wheatgrass was harvested with the help of stainless steel scissors (Plate 3.8). Centrifugal Juicer was used to extract the wheatgrass juice (Plate 3.11). Wheatgrass of eight days maturity was used for juice extraction (Fig. 3.1).

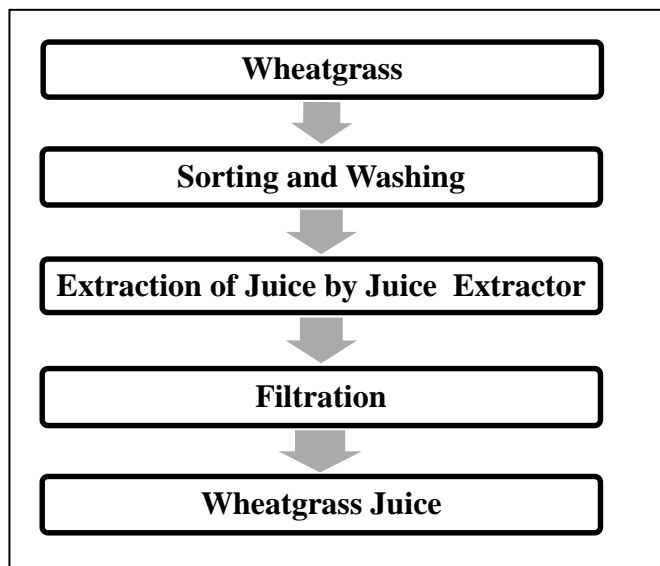


Fig. 3.1: Wheatgrass juice extraction process

Carrot juice was extracted from well matured carrots. The carrots were washed with tap water and peeled by peeler (Plate 3.9). The juice was extracted by juicer and filtered. The juice was kept under refrigerated condition till further use. The carrot juice (Plate 3.12) was obtained by following method given below (Fig. 3.2).

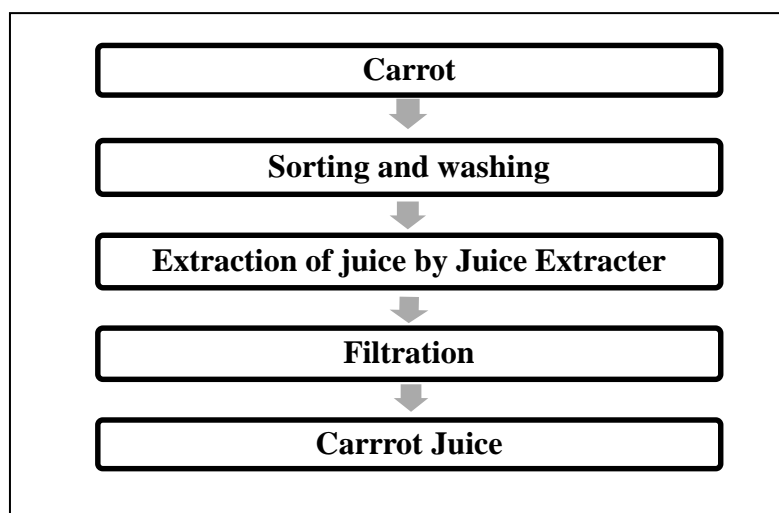


Fig. 3.2: Carrot juice extraction



Plate 3.11: Juice extraction



Plate 3.12: Extracted juices

3.5 Preparation of Wheatgrass and Carrot based Blend Juice

Wheatgrass and carrot-based mix juice was extracted as per method suggested by Eriq (2011). Juice prepared from wheatgrass and carrots (Plate 3.12) were blended by combining the two juices (Fig.3.3) according to the Table 3.1. The juice were pasteurized at (85°C for two minutes) after that juice were allowed to cool for some time and then kept at a refrigeration temperature in 200 ml clear plastic bottles Hasani *et al.*, (2021).

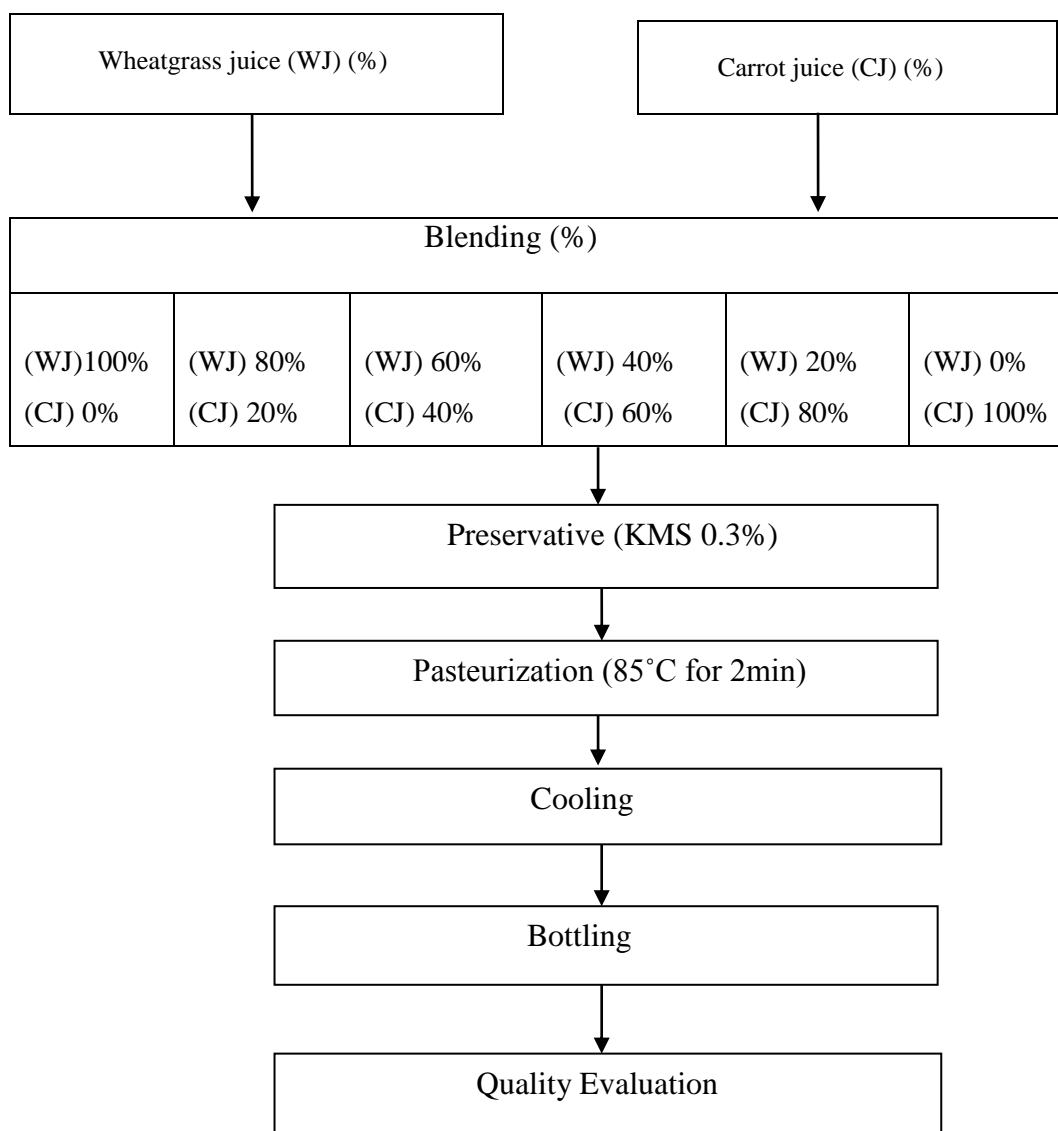


Fig.3.3: Blending of juice

Table 3.1 Variation of the wheatgrass juice and carrot juice in blended juice

Sample	Wheatgrass juice(WJ)	Carrot juice(CJ)
T ₀	100	0
T ₁	80	20
T ₂	60	40
T ₃	40	60
T ₄	20	80
T ₅	0	100

3.6 Physico-Chemical Analysis of Blended Juice

3.6.1 Moisture content

The moisture contents of the wheatgrass and carrot samples were calculated using the AOAC technique (2005). Ten grams of samples (w_1) were placed in a moisture box. The moisture box put in hot air oven (Plate 3.13) maintained at 105°C for 24 hours. The moisture box was removed from hot air oven and then placed into the desiccators for some time for cooling and also to avoid moisture absorption. After some time weight of dried samples were recorded as (w_2). The percentage of moisture content is calculated using given Eqn. (3.1)

$$\text{M. C. (db)} = \frac{(W_1 - W_2)}{W_2} \times 100 \quad \dots 3.1$$

Where,

M.C. (db) = Moisture content, (%)

W_1 = Initial weight of the sample, (g)

W_2 = Final weight of the sample, (g)



Plate: 3.13 Hot air oven

3.6.2 Total soluble solids (TSS)

The total soluble solid (TSS) is defined as the amount of sugar and soluble minerals present in fruits and vegetables. The method for its determination by means of a hand refractometer is given below (Plate 3.14).

Principle

A refractometer is based on the principle of total refraction, which describe the how light bends as it crosses the boundary between one medium and another.

Procedure

1. First inspection of the prism was done in order to ensure the surface is dry and clean.
2. Few drop of distilled water was applied with the help of pasture pipettes on the prism window.
3. Zero button was pressed. Instrument shows the reading 00 on the display after few second.
4. Prism window was cleaned with tissue paper to insure it is clean and dry.
5. Few drops of samples were applied with the help of pasture pipette onto the prism window.
5. Start button was pressed; the instrument (Plate 3.14) begins to measure.
6. After few second reading was displayed on the screen.



Plate 3.14: Digital refractometer

3.6.3 pH

The pH is the negative logarithmic measurement of the amount of hydrogen ions that are present in a solution. The pH is observation of acidity of solution. The pH is measured on a range of 0 to 14. The pH value of 7 is considered as neutral, meaning it is neither acidic nor basic. If the pH value is below 7, it is acidic and above 7 considered as a more base. Many chemical and biological processes depend significantly on the pH of the product to succeed. The pH also plays a crucial role in acceptance of product.

Principle

In a solution, hydrogen ions conduct an electric current. A potential difference forms across a very thin glass membrane that separates two solutions with different hydrogen ion concentrations one within and one outside the glass electrode when a glass electrode is submerged in a hydrogen-ion-containing solution. The pH meter monitors this potential difference and, using internal calibration, transforms it to a pH measurement that is displayed on a screen. It is used to measure pH on a scale from 0 to 14.

Procedure

1. First protective cap was removed from the pH probe.
2. Beaker was placed under the pH probe and temperature probe and both the probe were cleaned with distilled water. Beaker was removed and probes were dried with a tissue paper.
3. The pH meter was calibrated with the standard buffer solution of pH 4 and pH 7.
4. Probes were washed with distilled water and it dried with a tissue paper.
5. Sample was taken in to the beaker and probes were dipped into it.
6. Reading shown on the display of pH meter (Plate 3.15) for different samples which were used in the research.



Plate 3.15: pH meter

3.6.4 Ascorbic Acid (AOAC, 2005)

Ascorbic acid is also known as vitamin C. It is present in many fruits and vegetables. It is a water soluble vitamin. The following method was used to determine ascorbic acid:

Principle

Ascorbic acid transforms 2, 6-dichlorophenol indophenols dye to a colourless leucobase. Dehydroascorbic acid is produced by oxidizing ascorbic acid. The dye is a blue-colored compound, the final result appears pink.

Reagents

- 1. Oxalic acid 4%**
- 2. Dye solution:** Poured a little amount of distilled water containing 42 mg of sodium bicarbonate after that 52 mg, 2, 6-dichlorophenol indophenols dissolved in it and add distilled water to create the capacity 200 ml.
- 3. Stock standard solution:** 100 mg of ascorbic acid is dissolved in 100 ml of 4% oxalic acid in flask (1mg/ml).
- 4. Working standard:** 10 ml of stock solution was diluted with 100 ml of 4% oxalic acid.

Procedure

1. Working standard of 5 ml solution was pipette out and put in to 100 ml conical flask.
2. Ten ml of 4% oxalic acid was added and titrate against the dye (V_1 ml). Pink colour was appeared; the amount of dye used is equal to the amount of ascorbic acid.
3. Extracted sample 5 ml in 4% oxalic acid was made up to known volume (100 ml) and centrifuged.
4. Five ml of this supernatant and 10 ml of 4% oxalic acid (Plate 3.16) was pipette out and titrated against (Plate 3.17) the dye (V_2 ml).

Amount of Vitamin C was determined according to given Eqn. 3.2.

$$\text{Ascorbic acid} \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{0.5 \text{ mg}}{V_1\text{ml}} \times \frac{v_2}{5 \text{ ml}} \times \frac{100 \text{ ml}}{\text{weight of sample}} \times 100 \quad \dots 3.2$$



Plate 3.16: Prepared solution for ascorbic acid determination



Plate 3.17: Titration against dye with sample

3.6.5 Viscosity

Most fluids have some resistance to motion, which is referred to as "viscosity." When fluid layers move relative to one another, viscosity develops. It measures flow resistance that results from internal friction between the fluid layers as they pass one another during fluid flow. The concept of viscosity may also be used to describe a fluid's thickness or resistance to products going through it. Strong intermolecular forces lead a fluid with high viscosity to have a lot of internal friction, which prevents layers from moving past one another and opposes motion.

Working Principle

Brookfield viscometer (Plate 3.18) is rotational viscometer. For the measurement of the viscosity, measuring body (spindle) is immersed in the samples and rotated at a fixed speed. The force required to keep this speed constant, is a measurement for the viscosity.

Procedure

1. Viscometer was leveled referring to the bubble present on the top of the viscometer.
2. After leveling was done. Power was given to the viscometer.
3. After that cylindrical bar was attached to the viscometer, coupling nut was attached to the screw and then cylindrical sample adopter to the bar.
4. Ten ml of sample was taken in to sample holder and then it was inserted in the cylindrical sample adopter chamber.
5. Free hanging spindle was taken and attached to the coupling nut.
6. Two pipes were attached to the sample holder then it was put into the water bath in order to maintain the temperature of the sample.
7. Two modes were shown on the screen (external mode, stand alone mode). Stand alone mode was selected because viscometer was not attached to the computer. After the start button was pressed then auto zero was started displaying for few second. After the auto zero was completed different parameters were selected which were shown on the screen.
8. Motor was on button was pressed. Spindle was started rotating and after some time values were displayed on the screen.



Plate 3.18: Rapid viscometer

3.6.6 Colour

Colour is important for the consumer to judge quality based on its fundamental aesthetic value and food is no exception. The overall objective of food colour is to make it attractive and recognizable.

Principle

There were several colour scales used in a Hunter Lab Colorimeter (Plate 3.19) such as L^* , a^* and b^* which represents the surface colour. The colour values obtained as L^* is the lightness coefficient, ranging from 0 (black) to 100 (white), a^* is purple-red (positive a^* value) and blue-green (negative a^* value) and b^* , that represents yellow (positive b^* value) or blue (negative b^* value) colour (Plate 3.20) (McGuire, 1992).

Procedure

1. Power was given to the hunter lab calorimeter.
2. After that the instrument was calibrated with black as well as white standard plate.
3. Sample was placed at the light port (3.175 cm dia.) and start button was pressed
after few second reading was displayed on the screen (L^* , a^* , b^*).
4. Each sample was measured for colour values.

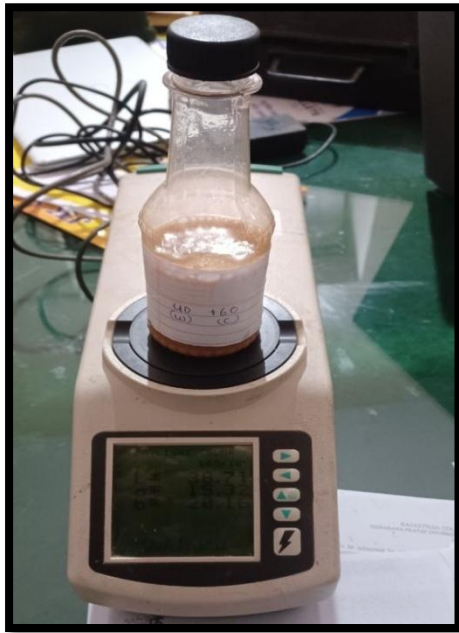


Plate 3.19: Hunter lab colorimeter

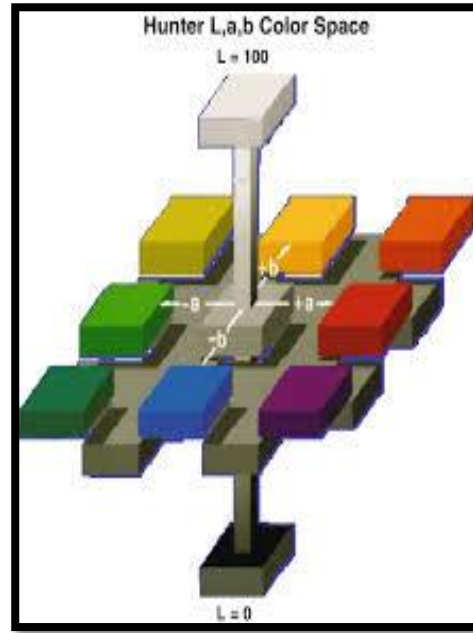


Plate 3.20: Colour scale presenting the relationship of colour index(L*,a*,b*)

3.6.7 β -carotene

The β -carotene content was determined using method suggested by Mishra and Gupta, (2016). A homogenous suspension was produced by weighing 10 ml of sample and dispersing it in 5 ml of water-saturated n-butanol. Samples were carefully mixed together and then left to stand 16 hours at room temperature in the dark. Once more combined and filtered using (Whatman No.1) into a volumetric flask of 100 ml. Using spectrophotometer (Plate 3.21), the optical density of the clear filtrate was determined at 440 nm. β -carotene was determined by given Eqn. 3.3:

$$\beta\text{-carotene content (PPM)} = 0.0105 + 23.5366 \text{ OD} \quad \dots 3.3$$

Where,

OD-Optical Density



Plate 3.21: Spectrophotometer used for β -carotene determination

3.7 Microbiological Analysis

Microbiological assessments of pasteurized juice samples were determined at 15 days interval for two months. In this study, the following dependent variables were chosen:

1. Standard plate count
2. Coliform bacteria count
3. Yeast and Mould

3.7.1 Serial dilution of samples

When doing bacteria count, bacterial colonies must be between 30 and 300 present on the plate. A minimum 30 bacterial colonies assures that data is statistically reliable; however, if there are more than 300 colonies, nutritional competition may restrict colony growth (Plate 3.24 d).

In microbiology, serial dilution is the standard method of sample dilution(Fig. 3.3).Utilizing the nutrient agar (NA), potato dextrose agar (PDA) and violet red bile agar (VRBA), respectively, one ml of the juice samples was examined for the population of total aerobic bacteria, yeast and mould and total coliforms.

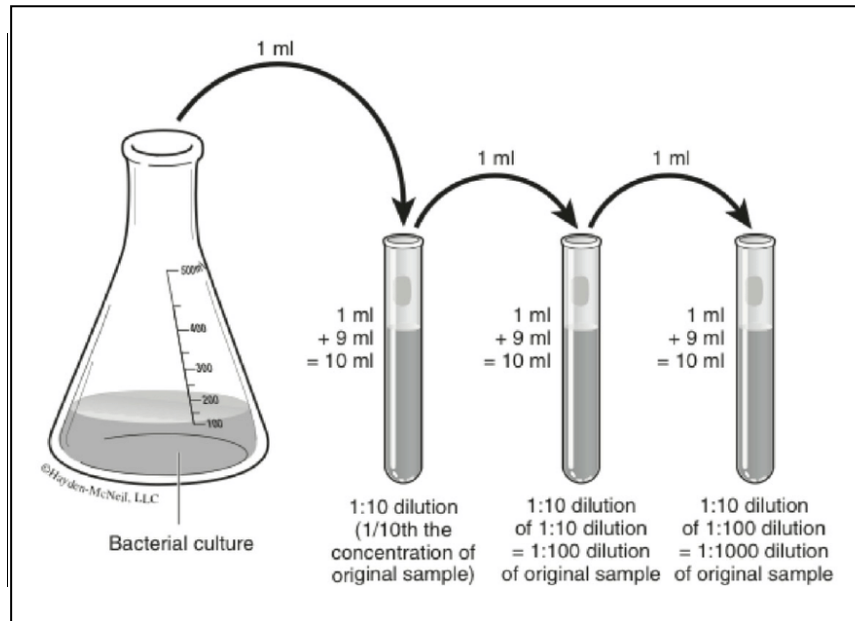


Fig 3.4: Serial Dilution of samples

3.7.2 Counting the colonies.

The colonies can be easily counted with the bacterial colony counters. Step followed for counting colonies are:

1. Place the plate upside down on the colony counter. Turn on the power switch; this will illuminate the plate from below (Plate 3.24 b).
2. Count the colonies while viewing through plate through the magnifying glass mounted above it.
3. Mark the petri plate over each colony with a marking pen to indicate which colonies have been counted. Use the hand tally provided to record the number of colonies counted.

3.7.3 Calculating the original cell concentration

The concentration of bacteria {Plate 3.24(b)} in the original samples was calculated by multiplying the colony counts by the total dilution factor. Only plate that contain between 30 and 300 colonies should be used to calculate the original concentration. The number of micro-organisms found in the samples were presented as the colonies forming unit per ml of sample.

$$\text{Total CFU} = \text{No. of colonies per volume plated} \times \text{dilution}$$

3.7.4 Analysis of standard plate count of bacteria

From the suitable dilution one ml of each sample prepared was used for plating in duplicates as described by Ranganna, (2001), and thereafter 15 ml of molten nutrient agar was poured aseptically to plates. The contents were mixed and plates were cooled. The plates were then inverted and incubated in an incubator

maintained at $37\pm 0.5^{\circ}\text{C}$ for 24 h and number of colony forming units (cfu/ml) were noted.

3.7.5 Analysis of yeast and mould

As described by Ranganna, (2001) one ml of suitable dilution from each sample prepared was used for plating in duplicates. After pouring the melted (~20 ml) VRBA (Plate 3.22) to each petri plates, the contents were mixed and agar was allowed to solidify. Again 5 ml of additional layer of VRBA was poured over the solidified later to have a second layer (Plate 3.24 a). The plates were inverted and incubated (Plate 3.23) at $37\pm 0.5^{\circ}\text{C}$ for 48 h and numbers of colony forming unit (cfu/ml) were noted.

3.7.6 Analysis of coliform bacteria

As described by Ranganna, (2001) one ml of suitable dilution from each sample prepared was used for plating in duplicates and thereafter 15 ml of molten PDA agar was poured aseptically to plates. The contents were mixed and plates were cooled. The plates were inverted and incubated {Plate 3.24(c)} at $25\pm 0.5^{\circ}\text{C}$ for 72 hour and numbers of colony forming unit (cfu/ml) were noted (Plate 3.24 c).



Plate 3.22: Different media cultures



Plate 3.23: Samples placed in incubator



(a) Laminar Air Flow



(b) Colony Counting Machine



(c) Incubator



(d) Autoclave

Plate 3.24: Equipments used in microbial analysis

3.8 Sensory Analysis

Food consumption and food quality are intimately related. Small and medium-sized processing companies are being compelled to think more and more about producing high-quality commodities. One of the producer's main instruments for positioning is quality, which is typically thought of as a degree of perfection and is important for marketability and customer pleasure. The assortment of a product's attributes has a significant role in determining the consumer's acceptance of the product. In order for food to maintain its quality and nutritional value along the food chain, its integrity and safety must be protected. The sensory analysis of blended juice

was evaluated for taste colour, flavor and overall acceptability. A nine-point Hedonic scale was used by panel of judges (Amerine *et al.*, 1965).

Table 3.2 Sensory score of juice samples

Sensory score	Rating
9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	Neither like nor dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike very much
1	Dislike extremely

3.9 Statistical Analysis

All the data obtained after experiment were statistically analysed using mean standard error, ANOVA two way annalysis as per standard method (Mehra *et al .*, 2017).

CHAPTER IV

RESULTS AND DISCUSSION

This chapter presents the outcomes of the study "Development of Wheatgrass and Carrot Blended Juice". The study was to develop process of preparation of juice by blending wheatgrass and carrot juice. The main objective of the experiment was to suggest blending combination and assess the quality of the blended juice and to determine its shelf life. Selected physico-chemical characteristics were determined to evaluate quality. These characteristics provided information about nutritional value of the blended juice. The storage study was conducted over a span of 60 days, with evaluations carried out at regular intervals of 15 days. The juice samples were stored at temperature of (4°C±1). The findings are presented and discussed under the following heading:

4.1 Physical parameters of wheatgrass and carrot

4.2 Development of wheatgrass and carrot blended juice

4.3 Sensory evaluation of juice samples

4.4 Quality evaluation of developed juice

4.5 Storage study of developed juice

4.6 Microbial load

4.1: Physical Parameters of Wheatgrass and Carrot

The leaves of wheatgrass, grown in the field, exhibited a bright green color, indicating the presence of chlorophyll, the length of wheatgrass varied from 15 to 20 centimeters. The shape of the wheatgrass was long and tapered. It was found to have a moisture content of 78.23%. Similar findings were reported by Ashok, 2011. The carrots were orange in colour and approximately 12 to 14 cm in length and diameter ranging from 3 to 4 cm. The moisture content was 86.48%, which is similar to the result reported by Sharma *et al.*, 2012.

4.2: Development of Wheatgrass and Carrot Blended Juice

Different combinations of wheatgrass and carrot juice were used to prepare blended juices. Two juice formulations were prepared: T₀ (100% wheatgrass juice) and T₅ (100% carrot juice) and four blends of wheatgrass and carrot juice were made by mixing them in different combination such as: 80:20 (T₁), 60:40 (T₂), 40:60 (T₃) and 20:80 (T₄), as outlined in Table 4.1. The prepared juice samples were pasteurized at a temperature of 85°C for 2 minutes and then stored in 200 ml transparent plastic bottles. The bottles were stored in fridge for 60 days. The juice formulations did not contain any artificial flavors, colors or thickeners. The physico-chemical analyses were conducted at 15 days interval for various parameters such as total soluble solids (TSS), pH, viscosity, β -carotene content colour and Vitamin-C content. The quality of the blended juice was evaluated.

Table 4.1: Variation of the wheatgrass and carrot in blended juice

Samples	Wheatgrass juice (WJ) (%)	Carrot juice (CJ) (%)
T ₀	100	00
T ₁	80	20
T ₂	60	40
T ₃	40	60
T ₄	20	80
T ₅	00	100



(a) T₀, (WJ)100:(CJ)00 (b) T₁, (WJ)80:(CJ)20 (c) T₂, (WJ)60:(CJ)40



(d) T₃, (WJ)40:(CJ)60 (e) T₄, (WJ)20:(CJ)80 (f) T₅, (WJ)00:(CJ)100

Plate 4.1: Blended juice samples prepared by various compositions of wheatgrass juice (WJ) and carrot juice (CJ) per cent

4.3: Sensory Evaluation of Juice Samples

The sensory characteristics of juice samples such as colour, flavor, taste and overall acceptability were analyzed by the panel of 15 judges and the mean sensory scores of various combinations of juice samples were determined.

4.3.1: Colour

Fig. 4.1 shows the mean sensory scores for the color of the juice samples of various proportions. The sensory ratings for colour were 8.10, 6.20, 6.80, 7.89, 7.00 and 6.9 for various samples wheatgrass juice at 100% (T₀), a blend of 80% wheatgrass juice and 20% carrot juice (T₁), a blend of 60% wheatgrass juice and 40% carrot juice (T₂), a blend of 40% wheatgrass juice and 60% carrot juice (T₃), a blend of 20% wheatgrass juice and 80% carrot juice (T₄) and carrot juice at 100% (T₅) respectively. The sample blend of 40% wheatgrass juice and 60% carrot juice (T₃), received the highest sensory score of 7.89 for colour, indicating the favorable combination among all the different juice blends. The ANOVA in Table 4.2 shows that mean sensory score for colour of the juice samples are significant at 5% level of significance.

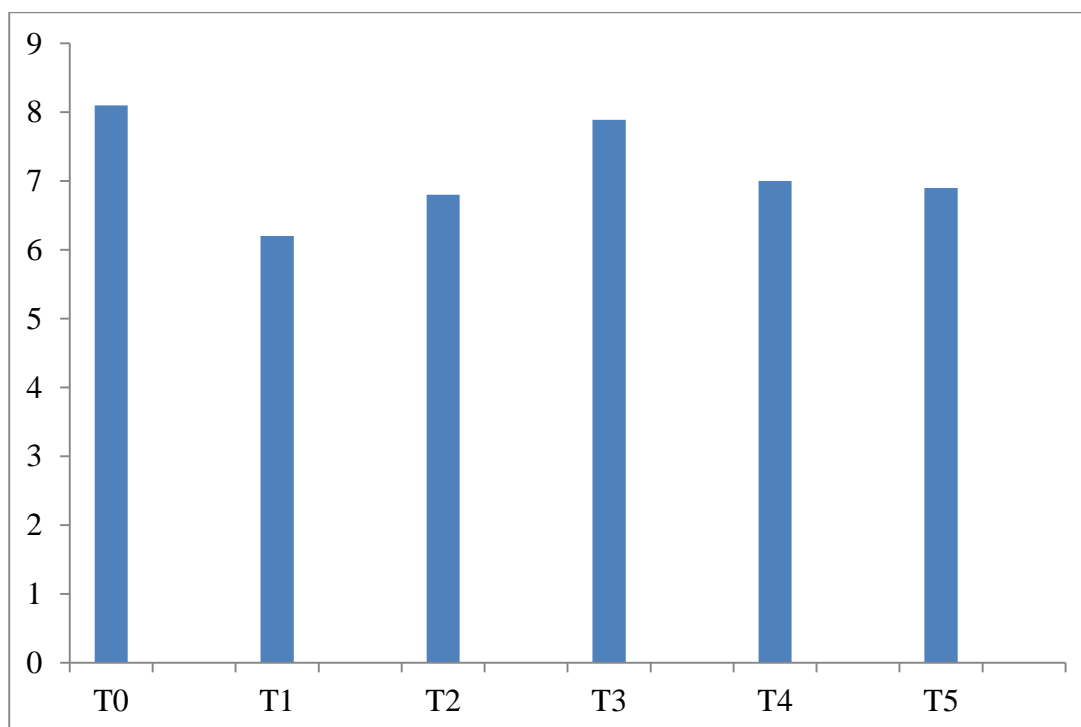


Fig. 4.1: Mean sensory score of colour for juice samples

Table 4.2: ANOVA for colour of juice samples

Source of Variation	SS	df	MS	F	P-value	F crit	Result	S.Ed	C.D at 5%
Rows	4.016667	15	1.778889	5.343636	0.03809	3.447063	S	0.04005	0.341
Columns	16.16647	1	8.083333	26.72727	0.00077	5.323253			
Error	1.433233	6	0.455556						
Total	24.91467	22							

S-significant

4.3.2: Flavor

The average sensory scores for the flavor of juice samples prepared with different combinations are presented in Fig 4.2. The samples consisted of wheatgrass juice at 100% (T₀), a blend of 80% wheatgrass juice and 20% carrot juice (T₁), a blend of 60% wheatgrass juice and 40% carrot juice (T₂), a blend of 40% wheatgrass juice and 60% carrot juice (T₃), a blend of 20% wheatgrass juice and 80% carrot juice (T₄) and carrot juice at 100% (T₅). The mean sensory scores for flavor were recorded as 5.10, 6.20, 6.40, 7.61, 7.50 and 7.40, for the sample T₀, T₁, T₂, T₃, T₄ and T₅ respectively. The sample labeled as T₃, which contained 40% wheatgrass juice and 60% carrot juice, received the highest sensory score of 7.61. The ANOVA in Table 4.3 shows that mean sensory score for flavor of juice samples are significant at 5% level of significance.

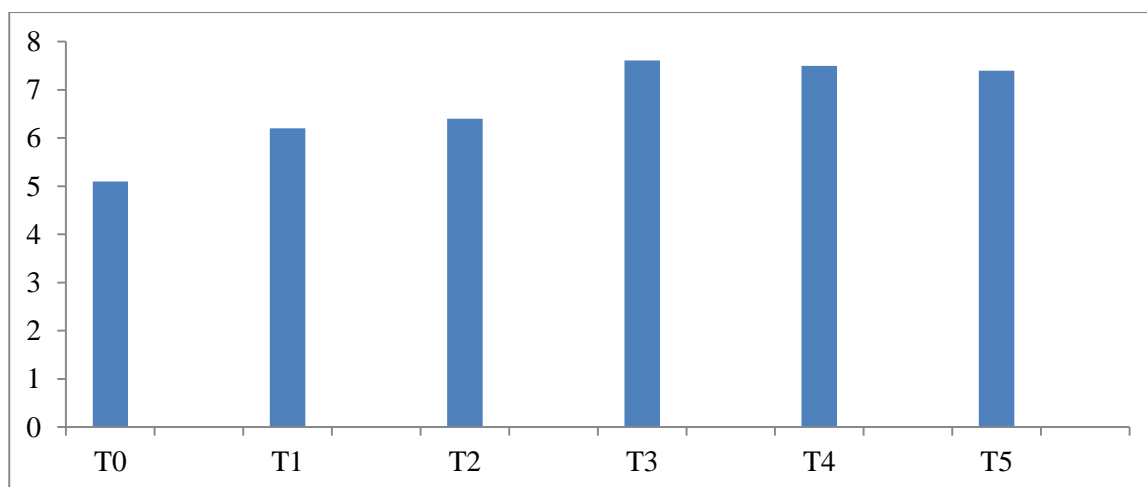


Fig. 4.2: Mean sensory score of flavor for juice samples

Table 4.3: ANOVA for flavor of juice samples

Source of Variation	SS	df	MS	F	P-value	F crit	Result	S.Ed	C.D at 5%
Rows	5.916667	15	1.538889	5.363636	0.03109	3.757063	S	0.03105	0.461
Columns	19.16667	1	8.083333	29.72727	0.00377	5.223253			
Error	1.732333	6	0.405556						
Total	22.91667	22							

S-significant

4.3.3: Taste

The samples consisted of various combination of wheatgrass and carrot juice such as wheatgrass juice at 100% (T₀), a blend of 80% wheatgrass juice and 20% carrot juice (T₁), a blend of 60% wheatgrass juice and 40% carrot juice (T₂), a blend of 40% wheatgrass juice and 60% carrot juice (T₃), a blend of 20% wheatgrass juice and 80% carrot juice (T₄), and carrot juice at 100% (T₅). The mean sensory rating (Fig.4.3) for the taste of the juice samples at various combinations were 6.00, 6.21, 6.81, 8.31, 8.18 and 8.13 for the sample T₀, T₁, T₂, T₃, T₄ and T₅ respectively. The blend (T₃) containing 40% wheatgrass juice and 60% carrot juice received the highest sensory score 8.31. The ANOVA in Table 4.4 shows that mean sensory score for taste of juice samples are significant at 5% level of significance.

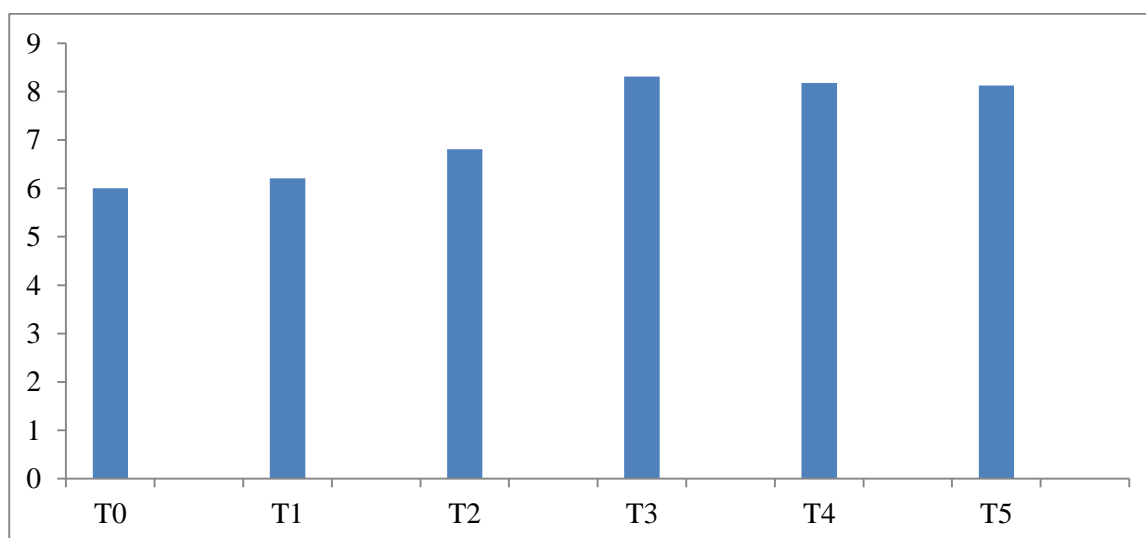


Fig. 4.3: Mean sensory score of taste for juice samples

Table 4.4: ANOVA for taste of juice samples

Source of Variation	SS	df	MS	F	P-value	F crit	Result	S.Ed	C.D at 5%
Rows	4.916667	15	1.778889	5.363636	0.03909	4.77063	S	0.03105	0.361
Columns	18.16667	1	7.093333	28.72227	0.00077	5.113253			
Error	1.833333	6	0.305556						
Total	24.91667	22							

S-significant

4.3.4: Overall acceptability

The mean rating of the overall acceptability for the different juice samples are presented in Fig.4.4. The samples included 100% wheatgrass juice (T₀), a blend of 80% wheatgrass juice and 20% carrot juice (T₁), a blend of 60% wheatgrass juice and 40% carrot juice (T₂), a blend of 40% wheatgrass juice and 60% carrot juice (T₃), a blend of 20% wheatgrass juice and 80% carrot juice (T₄) and carrot juice at 100% (T₅). The mean sensory ratings for overall acceptability were 5.51, 5.81, 6.89, 8.41, 8.11 and 8.01 for the sample T₀, T₁, T₂, T₃, T₄ and T₅ respectively. The blend (T₃) containing 40% wheatgrass juice and 60% carrot juice received the highest sensory score sensory score 8.41. The ANOVA in Table 4.5 shows that mean sensory score for Overall acceptability of juice samples are significant at 5% level of significance.

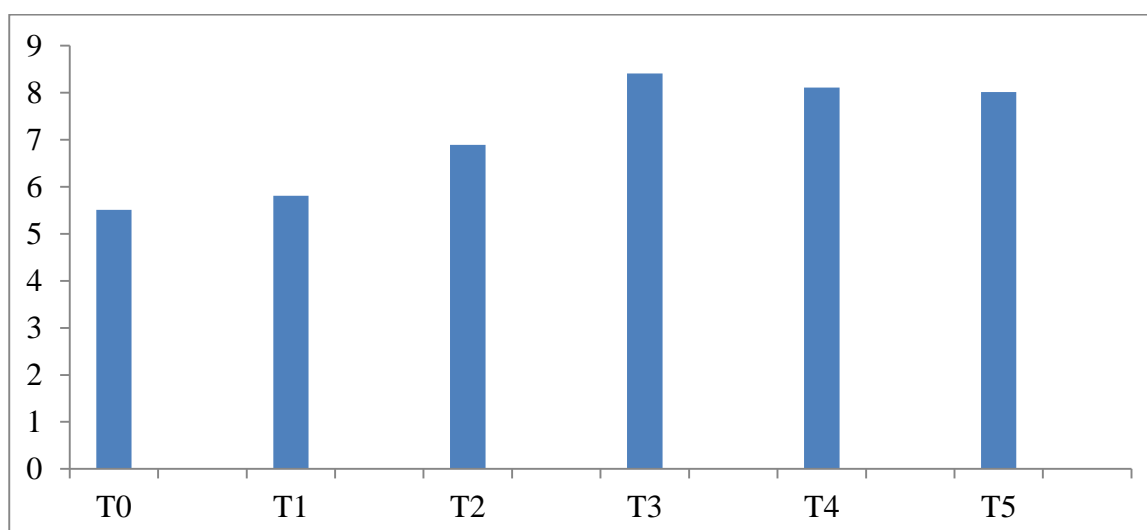


Fig. 4.4: Mean sensory score of overall acceptability for juice samples

Table 4.5: ANOVA for overall acceptability of juice samples

Source of Variation	SS	df	MS	F	P-value	F crit	Result	S.Ed	C.D at 5%
Rows	3.506667	15	1.638889	5.363636	0.03809	3.757043	S	0.03105	0.431
Columns	15.13367	1	8.083113	25.11727	0.00017	4.143233			
Error	1.211333	6	0.305116						
Total	22.91623	22							

S-significant

4.4: Quality Evaluation of Developed juice

The juice samples T₀, T₁, T₂, T₃, T₄ and T₅ were developed using different proportions of wheatgrass and carrot juice underwent sensory evaluation. After the sensory evaluation, it was found that sample containing 40% wheatgrass juice and 60% carrot juice (T₃) had the best result having highest sensory score of 7.89 for colour, flavor 7.61, taste 8.31 and overall acceptability 8.41. Therefore, a sample blend of 40% wheatgrass juice and 60% carrot juice (T₃) was selected for further quality evaluation and was also compared with quality of 100% wheatgrass juice (T₀) and 100% carrot juice (T₅).

4.4.1: Physico-chemical characteristics of wheatgrass and carrot juice

The physico-chemical characteristics of wheatgrass and carrot juice, as determined in the study, are presented in Fig. 4.6. The pH of sample 100% wheatgrass juice (T₀), 40% wheatgrass juice and 60% carrot juice (T₃) and 100% carrot juice (T₅) shows the value of 4.30, 4.51 and 4.71 respectively. This change in pH could be attributed to an increase in titrable acidity, as acidity and pH are known to have an inverse relationship, as explained by Jan and Masih, 2012. Similarly total soluble solid contents were 5.30, 7.12 and 8.10°Brix for 100 % wheatgrass juice (T₀), a blend of 40% wheatgrass juice and 60% carrot juice (T₃) and 100% carrot juice (T₅) respectively. According to Jingfei and Vasantha, 2012 the alterations might have been caused by the inversion of sucrose into glucose and fructose. Vitamin-C content of different samples by 100% wheatgrass juice (T₀), 40% wheatgrass juice and 60% carrot juice (T₃) and 100% carrot juice (T₅) were 25.52, 14.17 and 4.21 mg/100ml respectively. This is due to the presence of oxygen; both enzymatic and non-enzymatic catalysts can facilitate the oxidation of ascorbic acid, leading to its

degradation. Similar results were also found by Jain and Masih, 2012. The β -carotene content of sample 100% wheatgrass juice (T_0), 40% wheatgrass juice and 60% carrot juice (T_3) and 100% carrot juice (T_5) were 2.30, 6.01 and 8.33 mg/100ml respectively. According to the Lavelli and Sereikaite, 2022 β -carotene changes due to the double bond breaks which lead to chemical reaction. The level of viscosity in the sample 100% wheatgrass juice (T_0), 40% wheatgrass juice and 60% carrot juice (T_3) and 100% carrot juice (T_5) were 1.20cP, 1.32cP and 1.40cP respectively. According to Mehmood, 2008 the inversion of sucrose into glucose and fructose may have contributed to the increase in juice viscosity.

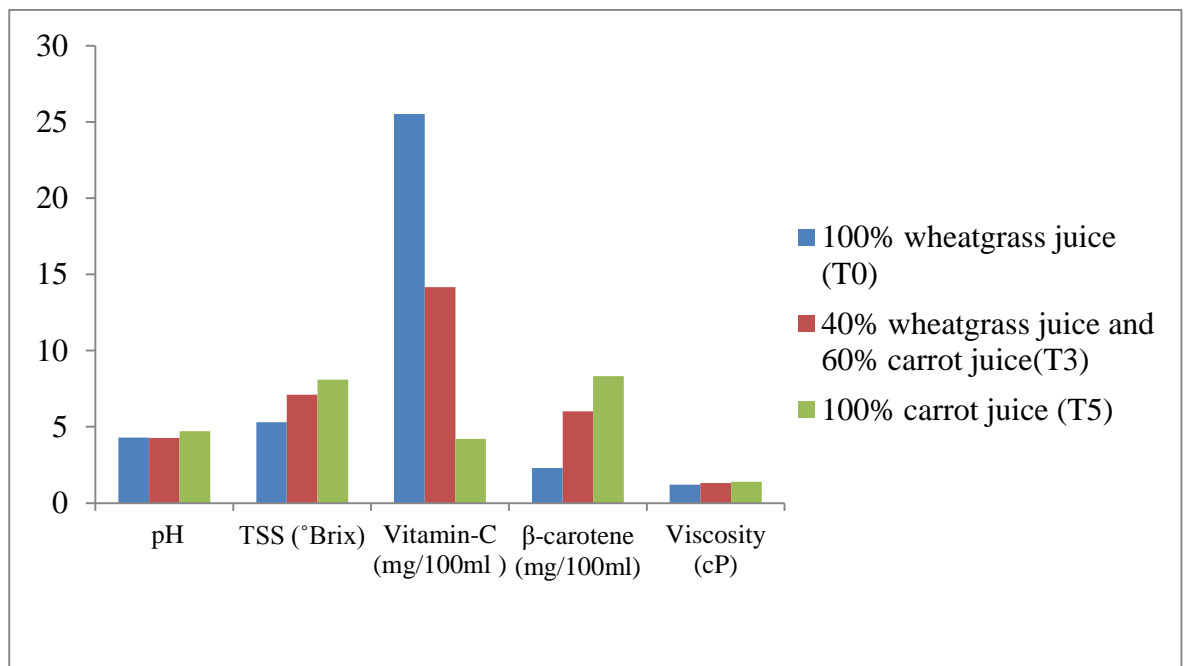


Fig. 4.5: Variation of Physico-chemical properties of juice

4.5: Storage Study of Developed Juices

A storage study of developed juices was conducted to assess its shelf life and quality. The study involved monitoring the juice's physical and chemical attributes during storage.

4.5.1: Effect on the physico-chemical properties of juice sample packed in a plastic bottles and kept at refrigerated temperature (4°C) during storage

Juices were prepared by combining wheatgrass and carrot juice. The Total Soluble Solids (TSS), pH, vitamin-C content, β -carotene content and viscosity levels of the samples 100% wheatgrass juice (T₀), 40% wheatgrass juice and 60% carrot juice (T₃) and 100% carrot juice (T₅) were determined. Subsequently, these samples were subjected to refrigeration storage (4°C) to observe the alterations in their physico-chemical properties.

4.5.2 Effect on pH content

During a 60 day period of storage, the pH levels of the blended juice 100% wheatgrass juice (T₀), 40% wheatgrass juice and 60% carrot juice (T₃) and 100% carrot juice (T₅) packaged in plastic bottles, were analyzed. The results of this study are presented in Table 4.6.

Table 4.6: Variation in pH during refrigerated storage

Juice combination	0 Days	15 Days	30 Days	45Days	60 Days
100% wheatgrass juice(T ₀)	4.30	4.21	4.12	4.01	3.91
40% wheatgrass juice and 60% carrot juice (T ₃)	4.51	4.41	4.31	4.22	4.11
100% carrot juice (T ₅)	4.71	4.61	4.52	4.41	4.29

In the period of 60 days, the pH values of the juice samples reduced for all formulations. Treatment 100% wheatgrass (T₀) exhibited decrease of 27.21% in pH, starting from 4.30 on the 0 days and reaching 3.91 after 60 days of storage at 4°C. Similarly, treatment 40% wheatgrass and 60% carrot juice (T₃) also showed a decrease in pH, albeit of lesser magnitude of 23.98%. The pH value decreased from 4.51 on the 0 days to 4.11 after 60 days. Treatment 100% carrot juice (T₅) had a pH value of 4.71 on the 0 days, which decreased to 4.29 after 60 days, resulting in a variation of 24.71%. These variations (Fig. 4.6) in pH indicate a gradual increase in acidity over the course of 60 days for all the treatments. The data of variation in pH

values during storage of 60 days were statistically analysed and regression equation of following linear form was predicated (4.1).

$$Y = mx + c$$

..4.1.

Where, Y is pH and X is the storage periods in days. The different values of regression coefficient of (m and c) along with R² of stastical analysis are presented in Table 4.7. The findings are consistent with the outcomes reported by Cecilia and Maia, 2002 in their study on apple juice. The decrease in pH value observed during storage could potentially be attributed to the degradation of pectin and the consequent release of free acids. A similar observation was also made by Muhammad (1986) in citrus fruit juices and by Zeb *et al.*, (2008) in grape juice. The obtained results were found to be significant at 5% level of significance from ANOVA in Table 4.8. According to the Food Safety and Standards Authority of India (FSSAI), the acceptable pH range for fruit and vegetable juices is in between 3.0 to 4.5.

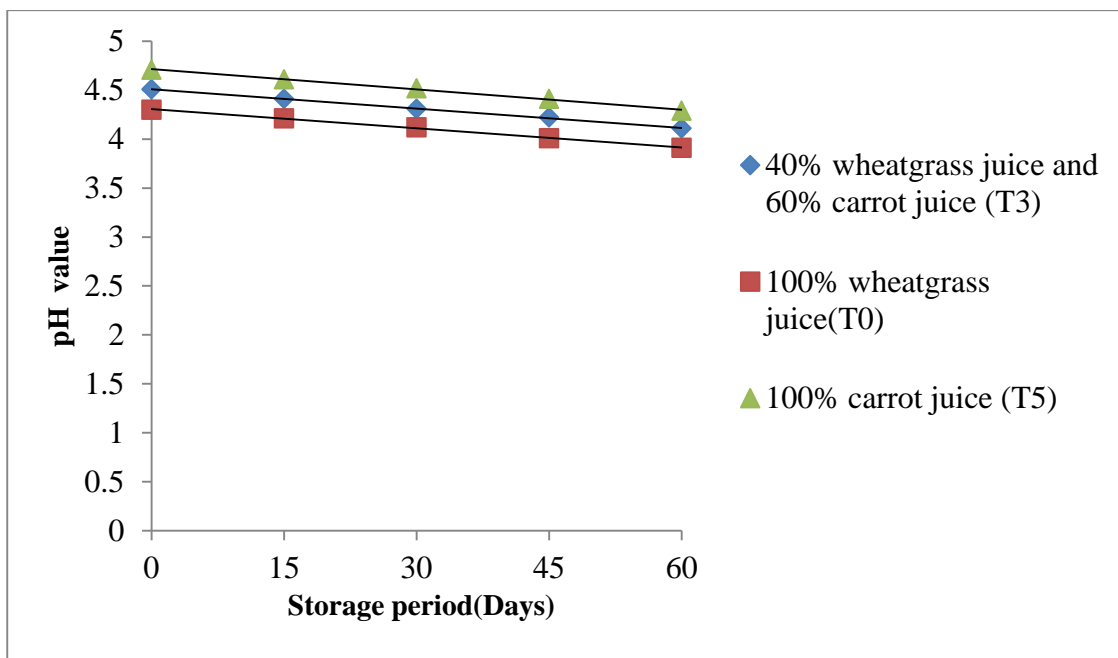


Fig. 4.6 Variation in pH for 100% wheatgrass juice (T₀), 40% wheatgrass juice and 60% carrot juice (T₃) and 100% carrot storage period

Table 4.7: Regression coefficients and R² values of Eqa. 4.1

Juice combination	m	c	R ²
100% wheatgrass juice(T ₀)	-0.101	3.919	0.997
40% wheatgrass juice and 60% carrot juice (T ₃)	-0.098	4.404	0.998
100% carrot juice (T ₅)	-0.104	4.82	0.997

Table 4.8: ANOVA showing effect of storage duration on pH of juice samples

Source of variation	SS	df	MS	F	P-value	F crit	Result	S.Ed	C.D at 5%
Rows	0.18636	3	0.062123	11.9195	0.00173	3.86258	S	0.0268	0.052
Columns	0.36451	5	0.121506	23.3136	0.00011	3.86258			
Error	0.04690	9	0.005212						
Total	0.59779	17							

S-significant

4.5.3 Effect on TSS content

The total soluble solids (TSS) contents in both standard juices (100% wheatgrass juice and 100% carrot juice) and blend juice (40% wheatgrass juice and 60% carrot juice) increased in storage and maximum values were observed after day 60 (Table 4.9).

Table 4.9: Variation in total soluble solids (°Brix) during refrigerated storage

Juice combination	0 Days	15 Days	30 Days	45 Days	60 Days
100% wheatgrass juice (T ₀)	5.30	5.40	5.49	5.61	5.71
40% wheatgrass juice and 60% carrot juice (T ₃)	7.01	7.11	7.21	7.31	7.43
100% carrot juice (T ₅)	8.10	8.20	8.31	8.48	8.59

The graph is plotted between TSS (Total Soluble Solids) and storage periods. The TSS values increased (Fig. 4.7) over the course of 60 days for all treatments. The per cent change in TSS from the initial value (0 days) to the subsequent time points (15, 30, 45 and 60 days) showed an increasing trend. In 100% wheatgrass juice (T₀) the TSS increased by 1.36%, 16.04%, 32.56% and 45.58% after 15, 30, 45 and 60 days of storage, respectively. Similarly for 40% wheatgrass and 60% carrot juice (T₃), the TSS increased by 3.13%, 7.14%, 8.96% and 17.07% after 15, 30, 45 and 60 days, respectively. For 100% carrot juice (T₅) the corresponding increases in TSS were 1.23%, 9.88%, 16.05% and 22.47% after 15, 30, 45, and 60 days, respectively. The data of variation in TSS values during storage of 60 days were statistically analyzed and regression equation of following linear form was predicated (4.2).

$$Y = mx + c$$

..4.2.

Where, Y is TSS and X is the storage periods in days. The different values of regression coefficient (m and c) along with R² of statistical analysis are presented in Table 4.10. The obtained results were found to be significant at 5% level of significance from ANOVA in Table 4.11. The results of the current study align with previous research conducted by Awsi and Masih (2012) on pineapple juice. The conversion of sucrose into glucose and fructose could potentially explain the observed increase in total soluble solids of blended juice during storage.

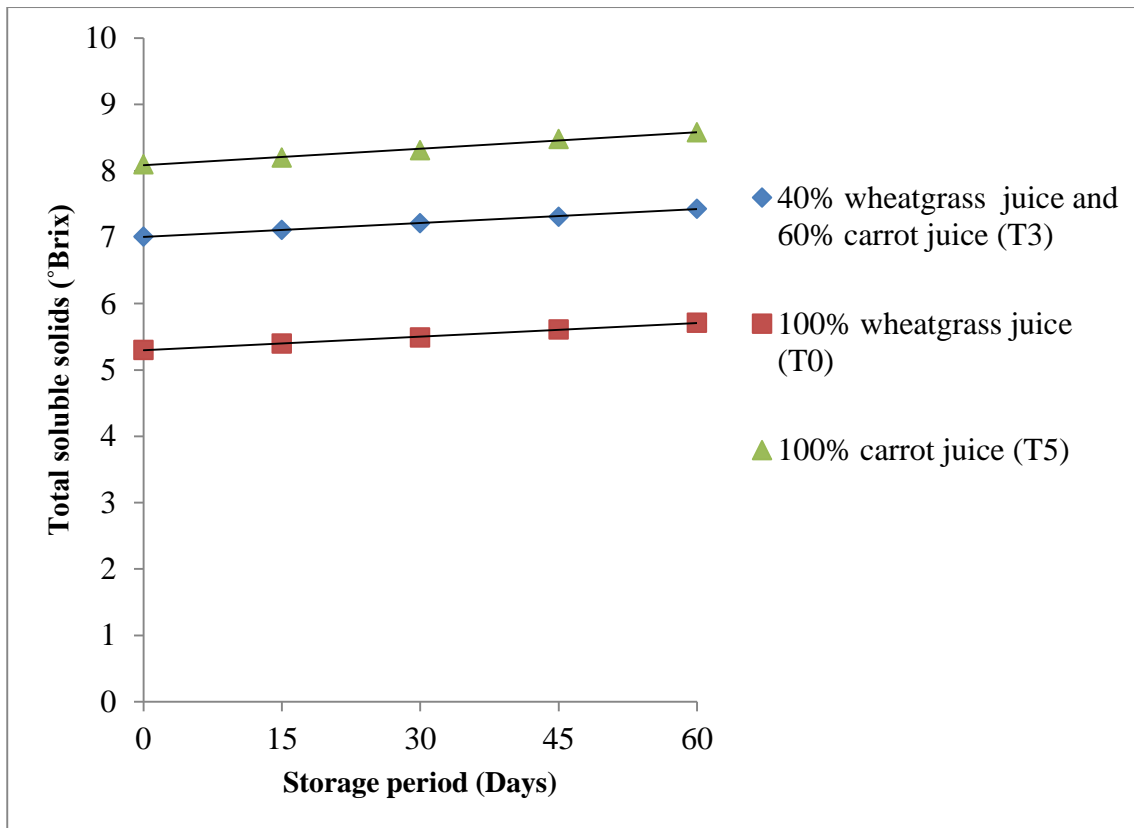


Fig 4.7 Variation in TSS for 100% wheatgrass juice (T₀), 40% wheatgrass juice and 60% carrot juice (T₃) and 100% carrot juice (T₅) with storage periods

Table 4.10: Regression coefficients and R² values of Eqa. 4.2.

Juice combination	m	c	R ²
100% wheatgrass juice(T ₀)	0.441	10.59	0.937
40% wheatgrass juice and 60% carrot juice (T ₃)	0.663	4.353	0.956
100% carrot juice (T ₅)	0.504	7.422	0.966

Table 4.11: ANOVA showing effect of total Soluble Solids on juice samples

Source of Variation	SS	df	MS	F	P-value	F crit	Result	S.Ed	C.D at 5%
Rows	2.390369	3	0.79679	193.51951	1.72E08	3.862548	S	0.138779	0.276
Columns	0.866219	5	0.28874	70.12734	1.46E06	3.862548			
Error	0.037056	9	0.004117						
Total	3.293644	17							

S-significant

4.5.4 Effect on Vitamin-C content

The study investigated the change in the vitamin-C contents during the storage of the samples of 100% wheatgrass juice (T₀), 40% wheatgrass juice and 60% carrot juice (T₃) and 100% carrot juice (T₅) for the period of 60 days and result are presented in Table 4.12. It provides a summary of the findings, which involved analyzing two standard samples (100% wheatgrass juice and 100% carrot juice) and one blended sample 40% wheatgrass juice and 60% carrot juice (T₃) at various storage times under refrigeration conditions.

Table 4.12: Variation in vitamin-C (mg/100ml) during refrigerated storage

Juice combination	0 Days	15Days	30Days	45Days	60 Days
100% wheatgrass juice (T ₀)	25.52	25.50	24.98	24.56	24.11
40% wheatgrass juice and 60% carrot juice (T ₃)	14.17	14.14	13.81	13.08	12.84
100% carrot juice (T ₅)	4.21	4.18	4.08	3.76	3.48

Fig 4.8 displays the variations in vitamin-C contents over a period of 60 days storage for different combinations. In Treatment 100% wheatgrass juice (T₀) vitamin-C content was 25.52 at 0 days and gradually decreased over time to 24.11 on 60th day. For Treatment 40% wheatgrass and 60% carrot juice (T₃), the initial value of vitamin-C content was 14.17 at 0 days and also decreased over time, reaching 12.84 on 60th day. In 100% carrot juice (T₅), the initial value was 4.21 at 0 days and decreased to 3.48 at 60th day. In 100% wheatgrass juice (T₀), the values decreased by 0.1% after 15 days, 2.1% after 30 days, 3.8% after 45 days, and 5.5% after 60 days. In 40% wheatgrass juice and 60% carrot juice (T₃), the values decreased by approximately 0.2% after 15 days, 2.5% after 30 days, 7.7% after 45 days and 9.4% after 60 days. For 100% carrot juice (T₅), the values decreased by 0.7% after 15 days, 3.1% after 30 days, 10.7% after 45 days and 17.3% after 60 days. Overall, the treatments exhibited a decline in vitamin-C content values over time. The data of variation in vitamin-C values during storage of 60 days were statistically analysed and regression equation of following linear form was predicated (4.3).

$$Y = mx + c \quad \dots 4.3$$

Where, Y is vitamin-c and X is the storage periods in days. The different values of regression coefficient regression coefficient (m and c) along with R² of stastical analysis are presented in Table 4.13. The obtained results were found to be significant at 5% level of significance as shown in ANOVA in Table 4.14. This decline in ascorbic acid, also known as vitamin C, can be attributed to the sensitivity of ascorbic acid to oxygen, light, and heat. In the presence of oxygen, both enzymatic and non-enzymatic catalyts can facilitate the oxidation of ascorbic acid, leading to its degradation. Similar results were also reported by Jan and Masih, 2012.

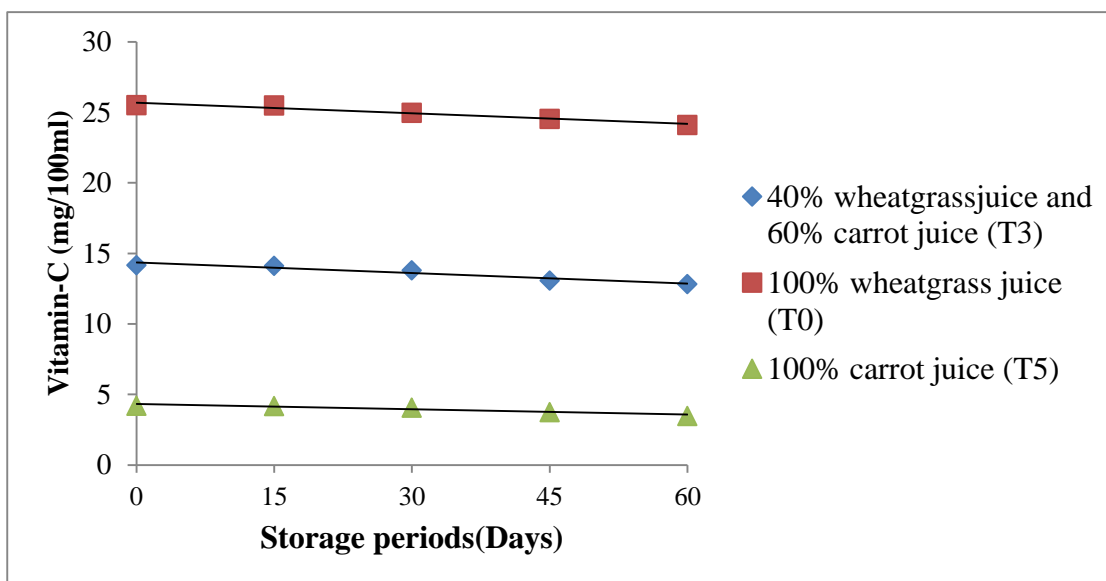


Fig 4.8 Variation in vitamin-C content for 100% wheatgrass juice (T₀), 40% wheatgrass juice and 60% carrot juice (T₃) and 100% carrot juice (T₅) with storage time

Table 4.13 Regression coefficients and R² values of Eq. 4.3.

Juice combination	m	c	R ²
100% wheatgrass juice(T ₀)	-0.372	14.72	0.917
40% wheatgrass juice and 60% carrot juice (T ₃)	-0.376	26.06	0.952
100% carrot juice (T ₅)	-0.188	4.406	0.896

Table 4.14: ANOVA showing effect of storage time on vitamin-C content juice samples

Source of Variation	SS	Df	MS	F	P-value	F crit	Result	S.Ed	C.D at 5%
Rows	0.001013	3	0.000338	28.390546	4.1E-05	3.862548	S	0.310120	0.0324
Columns	0.001835	3	0.000612	51.409815	4.9E-06	3.862548			
Error	0.000107	9	1.19E-05						
Total	0.002955	15							

S-significant

4.5.5: Effect on β -carotene

The β -carotene content in both the standard (100% wheatgrass juice and 100% carrot juice) and blended 40% wheatgrass juice and 60% carrot juice decreased with storage period (Fig 4.9). From the initial measurement at 0 days, 100% wheatgrass juice (T_0) exhibited a reduction of approximately 22.04% after 60th day. On the other hand, 40% wheatgrass and 60% carrot juice (T_3) had a decrease of around 9.31% during the same period and also 100% carrot juice (T_5) experienced percentage change of about 15.50% from 0 to 60th day. The data of variation in β -carotene values during storage of 60 days were statistically analyzed and regression equation of following linear form was predicated (4.4).

$$Y = mx + c \quad \text{..4.4.}$$

Where, Y is β -carotene and X is the storage periods in days. The different values of regression coefficient of (m and c) along with R^2 of statistical analysis are presented in Table 4.16. The maximum degradation of β -carotene was observed at day 60, according to the data presented in Table 4.15. The obtained results were found to be significant at 5% level of significance as mentioned in ANOVA in Table 4.17. After day 15 of storage, there was very little change in the β -carotene content. However, at days 30, 45 and 60 differences were observed, indicating the decrease in β -carotene levels over time. β -carotene is sensitive to degradation, particularly due to its many conjugated double bonds. Factors such as temperature, light and free radicals accelerate the degradation of β -carotene (Lavelli and Sereikaite , 2022).

Table 4.15: Variation in β -carotene (mg/100ml) during refrigerated storage

Juice combination	0 Days	15 Days	30Days	45 Days	60 Days
100% wheatgrass juice (T_0)	6.27	6.21	6.01	5.88	5.68
40% wheatgrass juice and 60% carrot juice (T_3)	2.30	2.20	2.11	2.01	1.78
100% carrot juice (T_5)	8.33	8.03	8.01	7.79	7.04

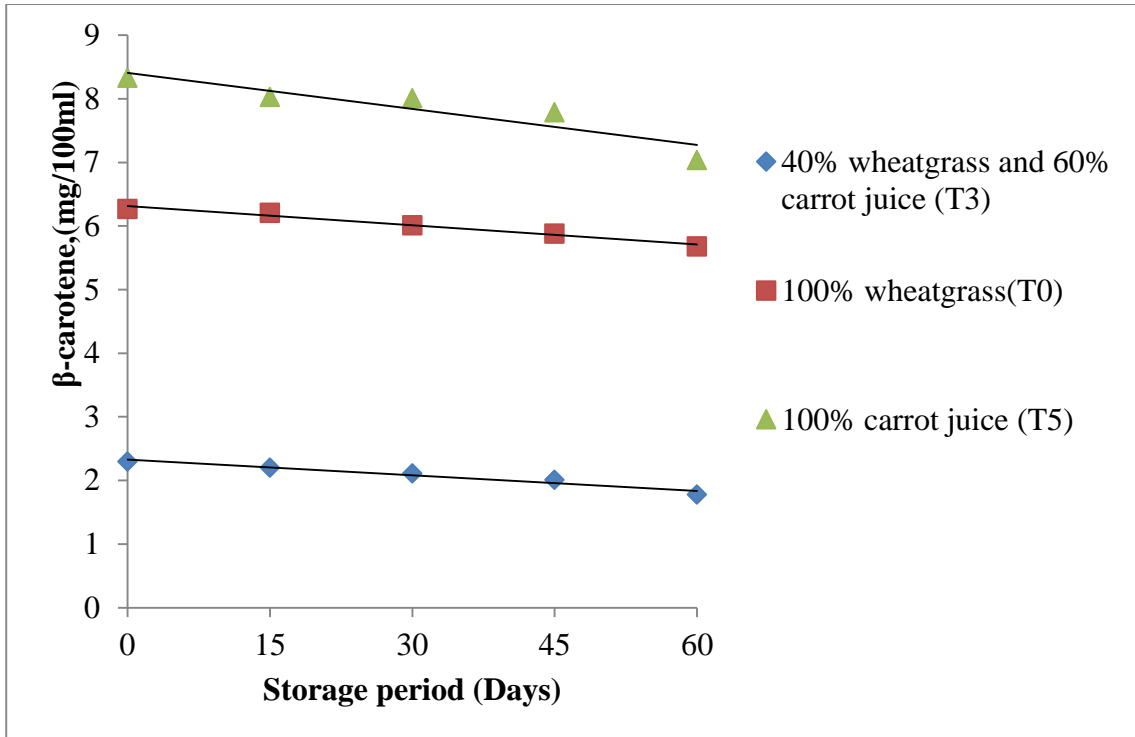


Fig 4.9 Variation in β -carotene content 100% wheatgrass juice (T₀), 40% wheatgrass juice and 60% carrot juice (T₃) and 100% carrot juice (T₅) with storage time

Table 4.16: Regression coefficients and R² values of Eqn. 4.4.

Juice combination	m	c	R ²
100% wheatgrass juice (T ₀)	-0.151	6.463	0.976
40% wheatgrass juice and 60% carrot juice (T ₃)	-0.123	2.449	0.953
100% wheatgrass juice (T ₀)	-0.151	6.463	0.976

Table 4.17: ANOVA showing effect of β -carotene content on juice samples

Source of Variation	SS	df	MS	F	P-value	F crit	Result	S.Ed	C.D at 5%
Rows	2.51195	3	0.837317	52.93888	4.85E-06	3.862548	S	0.0277	0.454
Columns	3.8816	5	1.293867	81.804	7.51E-07	3.862548			
Error	0.14235	9	0.015817						
Total	6.5359	17							

S-significant**4.5.6 Effect on viscosity**

Table 4.18 presents the variation in viscosity of the juices for the three combinations over a 60 days storage period. The data of variation in viscosity values during storage of 60 days were statistically analysed and regression equation of following linear form was predicated (4.5).

$$Y = mx + c$$

.4.5.

Where, Y is viscosity and X is the storage periods in days. The different values of regression coefficient of (m and c) along with R^2 of statistical analysis were presented in Table 4.19. The change in viscosity for the different treatments at the end of the 60days storage period falls within the following ranges: 1.20 to 1.52 cP for 100% wheatgrass juice (T_0), 1.32 to 1.61 cP for 40% wheatgrass and 60% carrot juice (T_3) and 1.40 to 1.62 cP for 100% carrot juice (T_5). The obtained results were found to be significant at 5% level of significance from ANOVA in Table 4.20. Fig. 4.10 provides a clear representation of how viscosity varies with storage duration for all developed juices. The figure demonstrates that the viscosity of all treatments progressively increased with storage. The increase in viscosity can potentially be attributed to the conversion of sucrose into glucose and fructose. Similar findings were reported by Mehmood, 2008 in their study on apple juice and Deshmukh *et al.*, (2015) in their research on sapota juice. This suggests that the inversion of sucrose into these simpler sugars may be possible reason to have contributed to the observed increase in viscosity of the juices.

Table 4.18: Variation in viscosity (cP) during refrigerated storage

Juice combination	0 Days	15Days	30Days	45Days	60 Days
100% wheatgrass juice (T ₀)	1.2	1.3	1.38	1.48	1.52
40% wheatgrass juice and 60% carrot juice (T ₃)	1.32	1.38	1.41	1.47	1.61
100% carrot juice (T ₅)	1.4	1.47	1.51	1.55	1.68

In the study, the variation in viscosity of all three treatments 100% wheatgrass juice (T₀), 40% wheatgrass juice and 60% carrot juice (T₃) and 100% carrot juice (T₅) were analysed on different days. For 100% wheatgrass juice (T₀), the viscosity increased gradually over time: it was 8.33% after 15th day, 15.00% after 30th days, 20.83% on 45th day and 26.67% after 60th day. Similarly, for 40% wheatgrass and 60% carrot juice (T₃), the viscosity also increased over time: it was 4.29% on 15th day, 8.57% at 30thday, 12.86% at 45th day, and 16.43% at 60th day and for 100% carrot juice (T₅) this variation was 5.00% at 15th day, 13.86% at 30th day, 20.71% at 45th day and 27.71% at 60th day.

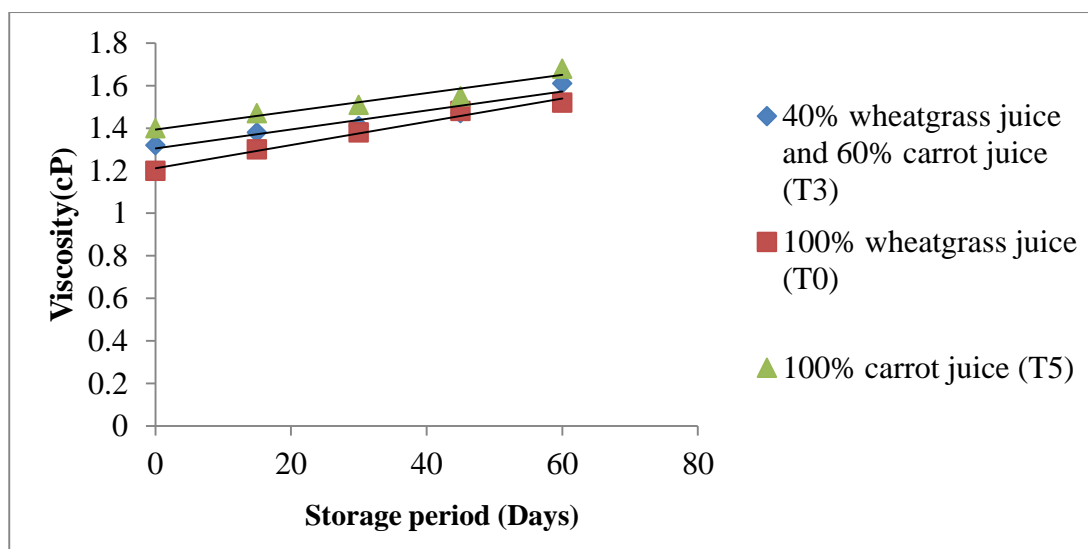
**Fig 4.10 Variation in viscosity for 100% wheatgrass juice (T₀), 40% wheatgrass juice and 60% carrot juice (T₃) and 100% carrot juice (T₅) with storage**

Table 4.19: Regression coefficients and R² values of Eqn. 4.5.

Juice combination	m	c	R ²
100% wheatgrass juice (T ₀)	0.049	1.117	0.963
40% wheatgrass juice and 60% carrot juice (T ₃)	0.054	1.138	0.948
100% carrot juice (T ₅)	0.038	1.336	0.989

Table 4.20: ANOVA showing effect of viscosity on juice samples

Source of Variation	SS	df	MS	F	P-value	F crit	Result	S.Ed	C.D at 5%
Rows	4.815283	3	1.605094	16.596794	9.96E-05	3.287382	S	0.2624	0.478
Columns	1.2265	5	0.2453	2.53642	0.074363	2.901295			
Error	1.450667	9	0.096711						
Total	7.49245	17							

S-significant**4.5.7 Effect of temperature and packaging on colour values of juice sample.**

The color values of juice samples, such as L*, a* and b* were determined using a Hunter Lab colorimeter (Plate 3.16). These values were recorded and presented in Table 4.12. The juice samples exhibited a range of values for the L* parameter, raising from 21.78 to 21.98. Similarly the colour value of a* and b* varying from -7.11 to -7.51 and 17.35 to 17.63 respectively (Table 4.21).

Table 4.21: Variation in colour during refrigerated storage

Sample		100% Wheatgrass juice (T ₀)	40% Wheatgrass juice and 60% carrot juice (T ₃)	100% Carrot juice (T ₅)
0 Days	L*	21.78	37.70	33.15
	a*	-7.11	8.06	7.88
	b*	17.35	16.32	12.49
15 Days	L*	21.80	37.76	33.18
	a*	-7.21	8.16	8.08
	b*	17.45	16.42	12.59
30 Days	L*	21.88	37.90	33.27
	a*	-7.31	8.26	8.17
	b*	17.35	16.52	12.69
45 Days	L*	21.94	38.01	33.45
	a*	-7.41	8.33	8.27
	b*	17.35	16.53	12.76
60 Days	L*	21.98	38.16	33.56
	a*	-7.51	8.46	8.96
	b*	17.63	17.02	12.89

Table 4.22 ANOVA showing L* value of juice samples

Source of Variation	SS	df	MS	F	P-value	F crit	Result	S.Ed	C.D at 5%
Rows	1.528369	3	0.69689	144.51951	1.78E-08	2.862581	S	0.168527	0.206
Columns	0.465969	3	0.48544	80.12134	1.66E-06	4.844548			
Error	0.047066	9	0.004017						
Total	2.292544	15							

S-significant

Table 4.23 ANOVA showing a* value of juice samples

Source of Variation	SS	df	MS	F	P-value	F crit	Result	S.Ed	C.D at 5%
Rows	5.527369	3	0.82679	158.5715	1.32E-02	4.822549	S	0.158379	0.281
Columns	0.766224	3	0.32874	60.12234	1.26E-01	2.862525			
Error	0.025056	9	0.004217						
Total	2.293454	15							

S-significant**Table 4.24 ANOVA showing b* value of juice samples**

Source of Variation	SS	df	MS	F	P-value	F crit	Result	S.Ed	C.D at 5%
Rows	4.391569	3	0.64619	153.5575	1.72E-08	3.862548	S	0.138779	0.276
Columns	0.766319	3	0.21874	70.12434	1.46E-06	3.862548			
Error	0.031056	9	0.003114						
Total	2.293554	15							

S-significant

4.6. Microbial load

The microbial analysis focused on three key parameters: the total plate count, yeast and mold count and coliform count. These parameters help to assess the microbial load and the presence of potentially harmful microorganisms in the juices. The total plate count refers to the number of viable microorganisms present in a given sample. It is an indicator of overall microbial growth and can provide insights into the hygiene and storage conditions of the juices. The count was measured in colony forming units per milliliter (cfu/ml). The yeast and mold count specifically targets the presence of yeast and mold colonies in the juice samples. Yeasts and molds can contribute to spoilage and affect the quality and safety of the juices. Similar to the total plate count, the yeast and mold count was measured in cfu/ml. The coliform count was determined to assess the presence of coliform bacteria in the juice samples. Coliforms are a group of bacteria that can indicate the potential presence of pathogens or fecal contamination. The count was also measured in cfu/ml. By analyzing the microbial counts over the 60-day storage period, valuable information can be obtained regarding the microbial stability and safety of the juices. During the 60-day storage

period, the deterioration of the juices was monitored by conducting microbiological tests. The results of these tests were analyzed and presented in Figures 4.11 and 4.12.

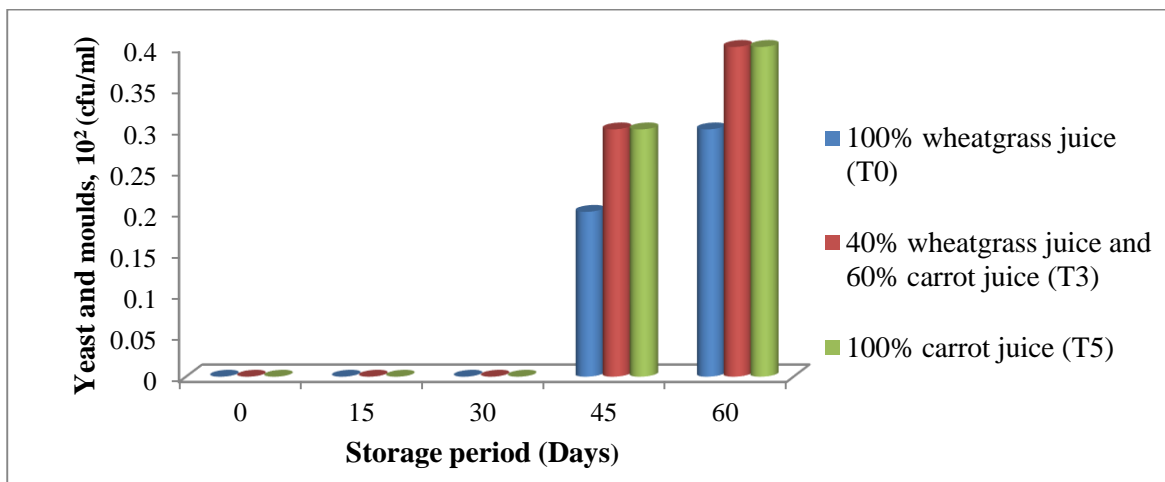


Fig. 4.11: Yeast and moulds growth for 100% wheatgrass juice (T₀), 40% wheatgrass juice and 60% carrot juice (T₃) and 100% carrot juice (T₅) with storage time

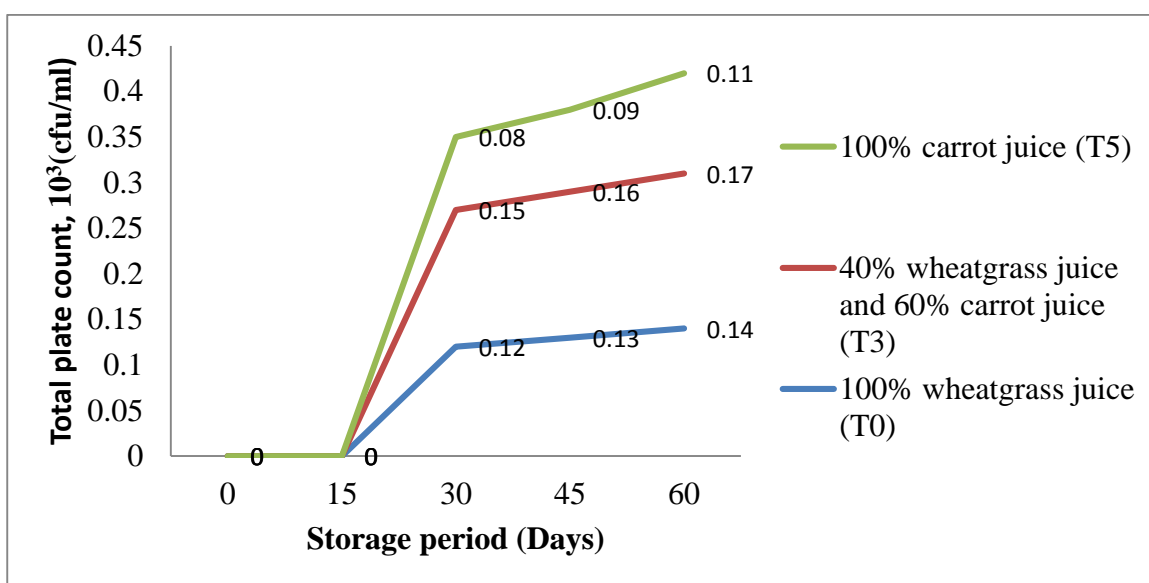


Fig. 4.12: Total plate count growth for 100% wheatgrass juice (T₀), 40%wheatgrass juice and 60% carrot juice (T₃) and 100% carrot juice (T₅) with storage time

On the first day, the total plate count, yeast and mold count, and coliform count of all the samples on the nutrient agar (NA), potato dextrose agar (PDA) and violet red bile agar (VRBA), respectively, were zero. This was because the juices had undergone pasteurization, where they were heated in a water bath at 85°C for 2 minutes. As a result, the product was deemed suitable for consumption.

Throughout the storage period, all samples remained free from coliform bacteria. In the initial 45 days, no yeast and mold growth was observed (Table 4.25) in any juice samples. However, after this period, some growth was noticed in all samples. ANOVA shows their level of significance at 5% level (Table 4.26 and Table 4.28). The observed growth remained within the safe limits for juices as specified by the regulations outlined in FSSAI-2019. According to these regulations, the growth of yeast and molds should be less than 1×10^3 cfu/ml.

Table 4.25: Yeast and moulds count during storage of juice

Days	Yeast and moulds, 10^2 (cfu/ml)		
	100% wheatgrass juice (T ₀)	40% wheatgrass juice and 60% carrot juice (T ₃)	100% carrot juice (T ₅)
0	0.0	0.0	0.0
15	0.0	0.0	0.0
30	0.0	0.0	0.0
45	0.2	0.3	0.3
60	0.3	0.4	0.4

Table 4.26: ANOVA showing effect of yeast and moulds count on juice samples

Source of Variation	SS	df	MS	F	P-value	F crit	Result	S.Ed	C.D at 5%
Rows	3.006413	5	1.002138	12.661110	0.0002183	3.287382	S	0.17876	0.356
Columns	0.761421	3	0.152284	1.9239740	0.1498022	2.901295			
Error	1.187263	15	0.079151						
Total	4.955096	23							

S-significant

Table 4.27: Total plate count during storage of juice samples

Days	Total plate count, 10 ³ (cfu/ml)			
	100% juice (T ₀)	wheatgrass	40% juice and carrot juice (T ₃)	wheatgrass and 60% carrot juice (T ₅)
0	0.00		0.00	0.00
15	0.00		0.00	0.00
30	0.12		0.15	0.08
45	0.13		0.16	0.09
60	0.14		0.1	0.11

Table 4.28: ANOVA showing effect of total plate count on juice samples

Source of Variation	SS	df	MS	F	P-value	F crit	Result	S.Ed	C.D at 5%
Rows	3.538833	3	1.179611	26.436173	0.09E-06	3.287382	S	0.16506	0.33
Columns	0.570183	5	0.114037	2.555666	0.072793	2.901295			
Error	0.669317	15	0.044621						
Total	4.778333	23							

S-significant

Over the course of the 60 days storage period, the total plate count value exhibited increase in all the juice samples, as presented in Table 4.27. However this increase remained within the acceptable limit specified by the Food Safety and Standards Authority of India (FSSAI) in their regulations concerning microbiological requirements for fruits, vegetables, and their products. According to the FSSAI, 2019 regulations on Process Hygiene Criteria, the acceptable limit for the total plate count is set at ($\geq 1 \times 10^4$) cfu/ml. Hence, the observed increment in the total plate count throughout the storage period adhered to the FSSAI guidelines.

CHAPTER V

SUMMARY AND CONCLUSIONS

In recent years, there has been an increasing interest in promoting healthy diets based on the consumption of fruits and vegetables. The consumption of health drinks is gaining popularity among people of all age groups due to their thirst-quenching, nutritional and medicinal properties. Various grasses are used in the beverage industry as health drinks, but wheatgrass stands out as the popular choice because of its numerous health benefits. Wheatgrass is rich in chlorophyll (70%), amino acids, minerals, vitamins and enzyme. Wheatgrass juices provide an opportunity to develop a blended juice by combining it with other juice. Blending wheatgrass and carrot juice has shown better levels of β -carotene and vitamins, making it a highly nutritious option as a food supplement. This blend prevents various health issues and improves immunity. Wheatgrass even referred to as "green blood" due to its ability to enhance red blood cell production. Additionally, it helps lower blood pressure, boosts energy and provides efficacy against diabetes, detoxifies the body, aids in weight loss, possesses antioxidant properties, fights free radicals and promotes healthy skin. Carrot juice, on the other hand, is a notable source of carotene and is valued for its vitamin and mineral content. With these points in mind, efforts have been made to develop a wheatgrass and carrot blended juice with the following objectives:

1. To develop the juice by blending of wheatgrass and carrot
2. To evaluate the quality of developed juice
3. To study the storage life of developed juice

Six juice formulations were prepared, 100% wheatgrass juice (T_0), a blend of 80% wheatgrass juice and 20% carrot juice (T_1), a blend of 60% wheatgrass juice and 40% carrot juice (T_2), a blend of 40% wheatgrass juice and 60% carrot juice (T_3), a blend of 20% wheatgrass juice and 80% carrot juice (T_4) and carrot juice at 100% (T_5). The juice samples were pasteurized and stored in transparent plastic bottles for 60 days. Sensory characteristics such as color, flavor, taste and overall acceptability were evaluated by a panel of 15 judges. These combinations of wheatgrass and carrot juice were analysed for their physico-chemical characteristics. Microbiological tests

were conducted to assess the total plate count, yeast and mold count and coliform count of the juice samples.

The major findings of the study are summarized as follows:

1. Physical Parameters of Wheatgrass and Carrot:

Wheatgrass leaves exhibited a bright green color, with a length ranging from 15 to 20 centimeters and a moisture content of $78.23\% \pm 0.5$. Carrots had an orange color, with a length of approximately 12 to 14 cm and diameter ranging from 3 to 4 cm and a moisture content of $86.48\% \pm 0.48$.

2. Sensory Evaluation of Juice Samples:

The juice samples 100% wheatgrass juice (T₀), a blend of 80% wheatgrass juice and 20% carrot juice (T₁), a blend of 60% wheatgrass juice and 40% carrot juice (T₂), a blend of 40% wheatgrass juice and 60% carrot juice (T₃), a blend of 20% wheatgrass juice and 80% carrot juice (T₄) and carrot juice at 100% (T₅). The blend containing 40% wheatgrass juice and 60% carrot juice (T₃) received the highest sensory scores for colour 7.89, flavor 7.61, taste 8.31 and overall acceptability 8.41 among six blended juices.

3. Storage Study of Developed Juice:

The sample blend of 40% wheatgrass juice and 60% carrot juice (T₃) was selected for further quality evaluation along with 100% wheatgrass juice (T₀) and 100% carrot juice (T₅). The physico-chemical properties of the juice samples were monitored for 60-day storage period at refrigerated temperature (4°C). The pH levels decreased over time in all the juice combinations 100% wheatgrass juice (T₀), a blend of 80% wheatgrass juice and 20% carrot juice (T₁), a blend of 60% wheatgrass juice and 40% carrot juice (T₂), a blend of 40% wheatgrass juice and 60% carrot juice (T₃), indicating an increase in acidity.

The TSS content and viscosity increased in all the juice combinations during storage due to the conversion of sucrose into glucose and fructose during storage. No coliform bacteria were detected throughout the storage period. Yeast and mold growth remained within the safe limits specified by

regulations. The total plate count increased over time but remained within acceptable limits

The vitamin-C and β -carotene contents decreased in all the samples during storage. The decline in vitamin-C and β -carotene content in the juice samples can be attributed to the sensitivity of ascorbic acid to factors such as oxygen, light and heat.

Conclusion

The blend containing 40% wheatgrass juice and 60% carrot juice (T₃) is the best sample.

SUGGESTIONS FOR FUTURE WORK

1. Storage study at ambient temperature and refrigerated temperature may be conducted and compared.
2. Analysis of change in quality of juice samples packaged in a different material may be conducted.

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ABSTRACT IN ENGLISH

The popularity of wheatgrass and carrot juices has been on the rise due to the increasing awareness of its potential health benefits. This study aims to investigate the nutritional composition and sensory evaluation of the wheatgrass and carrot blended juice. Six different juice combinations were prepared, including 100% wheatgrass juice (T₀), a blend of 80% wheatgrass juice and 20% carrot juice (T₁), blend of 60% wheatgrass juice and 40% carrot juice (T₂), blend of 40% wheatgrass juice and 60% carrot juice (T₃), blend of 20% wheatgrass juice and 80% carrot juice (T₄), and 100% carrot juice (T₅). In the sensory evaluation, each of the juice combinations (T₀ to T₅) was assessed for taste, flavor, and overall acceptability. The results revealed that the blend containing 40% wheatgrass juice and 60% carrot juice (T₃) received the highest scores in all three sensory attributes, indicating its superior sensory profile compared to the other blends. Consequently, the 40% wheatgrass juice and 60% carrot juice (T₃) blend was selected for further storage assessment at 4°C for a period of 60 days along with T₀ and T₅. During the first 30 days of storage, the 40% wheatgrass juice and 60% carrot juice (T₃) blend demonstrated stability in its physicochemical properties, with low changes observed in pH and TSS (total soluble solids). These findings suggest that the overall quality of the blend remained well-maintained under refrigerated conditions during this initial period. However, beyond 30 days of storage, the 40% wheatgrass juice and 60% carrot juice (T₃) blend exhibited microbial growth but still in the permissible range.

अनुक्षेपण

व्हीटग्रास और गाजर के जूस की प्रसिद्धि ताजगी के पूर्वजागृति के कारण तेजी से बढ़ रही है। इस अध्ययन का उद्देश्य व्हीटग्रास और गाजर के मिश्रित जूस की पोषण संरचना, और इसकी सेंसरी मूल्यांकन का पता लगाना है। छः विभिन्न जूस के मिश्रण तैयार किए गए, जिनमें 100% गेहूं का जूस (T₀), 80% व्हीटग्रास और 20% गाजर का जूस का मिश्रण (T₁), 60% व्हीटग्रास और 40% गाजर का जूस का मिश्रण (T₂), 40% व्हीटग्रास और 60% व्हीटग्रास का मिश्रण (T₃), 20% व्हीटग्रास और 80% गाजर का जूस का मिश्रण (T₄) और 100% गाजर का जूस (T₅) शामिल थे। सेंसरी मूल्यांकन में, जूस के प्रत्येक मिश्रण (T₀ से T₅) का स्वाद, प्लेवर और संपूर्ण स्वीकार्यता के लिए मूल्यांकन किया गया। परिणाम ने दिखाया कि 40% व्हीटग्रास 60% गाजर का जूस का मिश्रण (T₃) ने तीनों सेंसरी गुणों में सर्वोत्तम अंक प्राप्त किए, जिससे इसके अन्य मिश्रणों की तुलना में इसकी उत्कृष्ट सेंसरी प्रोफाइल का पता चला। इसलिए, 40% व्हीटग्रास और 60% गाजर का जूस का मिश्रण (T₃) चयन किया गया था जिसे 4°C पर 60 दिनों के लिए T₀ और T₅ के साथ आगे के स्टोरेज मूल्यांकन के लिए चुना गया। पहले 30 दिनों के स्टोरेज के दौरान, 40% व्हीटग्रास और 60% गाजर का जूस का मिश्रण (T₃) ने अपने भौतिकीय-रसायनिक गुणों में स्थिरता दिखाई, जिसमें pH और TSS (कुल विलयनीय ठोस) में न्यूनतम परिवर्तन देखा गया। ये फिंडिंग्स सुझाव देते हैं कि इस आरंभिक अवधि के दौरान मिश्रण की समग्र गुणवत्ता को ठीक से बनाए रखा गया था। हालांकि, 30 दिनों से अधिक समय के स्टोरेज के बाद, 40% व्हीटग्रास 60% गाजर का जूस का मिश्रण (T₃) में माइक्रोबियल विकास में वृद्धि हुई परन्तु अनुमति सीमा के भीतर।

APPENDIX – A

List of equipment/instruments used

N o.	Instrument	Model	Specification	Applicati on
1.	Hot air oven	Khera Instrume nts	Size:605×910×455mm (3 trays),S.S chamber Temperature: 50 to 300°C	Moisture content determina tion
2.	Digital refractometer	RX- 5000α- Bev	Resolution Refractive index(nD)0.00001Brix:0.01% Te mperature: 0.01°C Accuracy: Refractiveindex (nD): ±0.00004 Brix : ±0.03% Temperature control range 5.00 to 60.00°C	To measure TSS value
3.	pH meter	PH2019	Measuring range: 0.00~14.00PH PowerSupply:4×1.5V(AG13) Dimensions: 175mm×30mm×22mm Resolution:0.001pH	To measure pH
4.	Weighing balance	BSA224 S-CW	Capacity : 5kg Readability:0.1g Uncertainty = 0.001%	To measure the weight of sample
5.	Spectrophoto mete	Lasany LI-2700	Double beam operating system λ = 190-1100 nm Power = AC 220V/50 Hz Weight = 25 kg Spectral band width = 1 nm	To measure absorbanc e value
6	Rapid viscometer	Brooke field DV- II	Viscosity range:0.1 to 100 cP Accuracy:±1% of range; Repeatability:±2.0% and rpm range:0.2 to 200 rpm	To measure viscosity
7.	Hunter lab colorimeter	Model no. - 45Serial no.- CXO- 787USA	-	To measure colour value
8.	Laminar Air flow	Laminar air flow	Air velocity: 0.45 m/s to 0.65 m/s Illumination: 1 or 2 LED Light Power supply: 220 volts /50 Hz	Laminar air flow is used for aseptic transfer of microbial culture

9.	Colony counter machine	S- 961	Magnification: 1.7X Dimension: 260X220X168 mm (LXBXH) Power Supply: 230V, ± 10% AC, 50Hz, 40 W	To count colonies of bacteria
10	Incubator	STXBI28	Temperature range: Ambient+10°C to 60°C Temperature accuracy: ±0.2°C @37°C Temperature controller: Dual display PID controller Display SV & PVAuto tuning feature Air circulation: Motorized blower / Axial fan Power supply: 220 Volts 50Hz	Provides a temperature controlled environment to support growth of microbiological cultures
11	Autoclave	LKPC010/30	Size-550×750 mm Capacity-187 Ltr Pressure 40 psi	To determine certain biological waste and sterilize media

APPENDIX - B

Table B: Performa for sensory evaluation of development of wheatgrass and carrot blended juice

Name:

Date:

Like extremely - 9

Like slightly - 6

Dislike moderately-3

Like very much- 8

Neither like nor dislike-5

Dislike very much-2

Like moderately-7

Dislike slightly-4

Dislike extremely-1

Sample	Taste	Flavour	Color	Overall acceptability
T₀				
T₁				
T₂				
T₃				
T₄				
T₅				

Signature:

Name:

APPENDIX - C

Table C: Score of sensory evaluation of juice

Sensory Attributes	Treat-ments	No. of panellists														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Colour	T ₀	9	8	8	7	8	9	8	8	7	8	7	7	8	9	8
	T ₁	9	8	9	8	7	9	8	7	9	8	8	8	9	7	8
	T ₂	8	8	7	9	7	8	8	9	9	7	8	7	9	7	9
	T ₃	8	7	8	9	8	9	8	9	7	8	9	7	9	8	7
	T ₄	8	7	8	7	8	9	7	8	9	7	8	9	7	8	9
	T ₅	9	8	9	8	7	9	8	7	9	8	8	8	9	7	8
Flavour	T ₀	6	5	6	5	6	4	6	5	4	6	5	5	6	5	5
	T ₁	7	8	9	8	7	9	8	7	9	8	8	8	9	7	8
	T ₂	7	8	7	9	7	8	8	9	9	7	8	7	9	7	9
	T ₃	8	7	8	9	8	9	8	9	7	8	9	7	9	8	7
	T ₄	8	7	7	7	7	8	7	7	8	8	7	7	8	8	9
	T ₅	9	8	9	8	8	8	7	7	9	7	8	8	9	7	8
Taste	T ₀	6	5	6	5	6	6	5	7	6	4	5	5	6	5	6
	T ₁	7	7	8	9	8	9	8	9	7	8	9	7	9	8	7
	T ₂	7	9	7	7	7	8	7	7	8	8	7	7	8	8	9
	T ₃	8	8	9	8	8	8	7	7	9	7	8	8	9	7	8
	T ₄	8	7	9	8	7	9	8	7	9	8	8	8	9	7	8
	T ₅	8	8	7	9	7	8	8	9	9	7	8	7	9	7	9
Overall Acceptability	T ₀	4	5	6	4	5	6	5	7	5	5	5	6	4	5	4
	T ₁	6	8	7	9	7	8	8	9	9	7	8	7	9	7	9
	T ₂	7	7	8	9	8	9	8	5	7	8	9	7	7	8	7
	T ₃	9	9	7	7	7	8	7	7	8	8	7	7	8	8	9
	T ₄	8	8	9	8	8	8	7	7	9	7	8	8	9	7	8
	T ₅	8	8	9	8	7	9	8	7	9	8	8	8	9	7	8

Appendix D

*Table 4A: Microbiological Standards for Fruits and Vegetables and their Products – Process Hygiene Criteria

Sl. No.	Product description ¹	Aerobic Plate Count				Yeast and Mold Count				<i>Enterobacteriaceae</i>				<i>Staphylococcus aureus</i> (Coagulase +ve)			
		Sampling Plan		Limit (cfu)		Sampling Plan		Limit (cfu)		Sampling Plan		Limit (cfu)		Sampling Plan		Limit (cfu)	
		n	c	m	M	n	c	m	M	n	c	m	M	n	c	m	M
1	Fresh ²	NA				NA											
2	Cut or minimally processed and packed, including juices (Non-thermally processed)	5	2	1x10 ⁶ /g	1x10 ⁷ /g	5	1	1x10 ⁷ /g	1x10 ⁴ /g	5	2	1x10 ² /g	1x10 ⁴ /g	5	1	1x10 ² /g	1x10 ³ /g
3	Fermented ³ or pickled or acidified or with preservatives	NA				5	1	1x10 ⁷ /g	1x10 ³ /g	5	2	1x10 ² /g	1x10 ³ /g	5	1	10/g	1x10 ² /g
4	Pasteurized Juices ⁴	5	2	1x10 ⁷ /ml	1x10 ⁹ /ml	5	1	1x10 ⁷ /ml	1x10 ³ /ml	5	0	Not detectable as per prescribed method		5	0	Absent/25ml	
	Carbonated Fruit beverages ⁴	5	1	50/ml	5x10 ³ /ml	5	0	<10/ml		5	0			5	0	Absent/25ml	
5	Frozen	5	2	4x10 ⁴ /g	5x10 ⁵ /g	5	1	1x10 ⁷ /g	1x10 ³ /g	5	2	1x10 ² /g	3x10 ² /g	5	1	20/g	1x10 ² /g
6	Dehydrated or dried	5	1	4x10 ⁴ /g	1x10 ⁵ /g	5	1	1x10 ⁷ /g	1x10 ⁴ /g	5	1	1x10 ² /g	1x10 ³ /g	5	1	10/g	1x10 ² /g
7	Thermally processed (other than pasteurization at less than 100°C)	5	1	1x10 ² /g	1x10 ³ /g	5	1	50/g	1x10 ² /g	5	0	Not detectable as per prescribed method		5	0	Absent/25g	
8	Retort processed ³	5	0	50/g		NA				5	0			5	0	Absent/25g	
	Test Methods ⁶	IS: 5402/ISO:4833				IS: 5403/ ISO 21527 Part 1 and Part 2				IS/ISO 7402/ ISO 21528 Part 2				IS:5887, Part 2 and IS 5887 part 8 (Sec 1) ISO 6888-1 or IS:5887 Part 8 (Sec2)/ISO 6888-2			

Appendix E

Table E 1: Variation of physico-chemical properties of juice

constituents	100% wheatgrass juice (T0)	40% wheatgrass juice and 60% carrot juice(T3)	100% carrot juice (T5)
pH	4.3	4.51	4.71
TSS (°Brix)	5.3	7.01	8.1
Vitamin-C (mg/100ml)	25.52	14.17	4.21
β-carotene (mg/100ml)	2.3	6.01	8.33
Viscosity (cP)	1.2	1.32	1.4

Table E 2: Variation in pH during refrigerated storage

Juice combination	0 Days	15 Days	30 Days	45Days	60 Days
100% wheatgrass juice(T ₀)	4.30	4.21	4.12	4.01	3.91
40% wheatgrass juice and 60% carrot juice (T ₃)	4.51	4.41	4.31	4.22	4.11
100% carrot juice (T ₅)	4.71	4.61	4.52	4.41	4.29
S.Ed			0.0268		
C.D at 5%			0.052		

Table E 3 Variation in total soluble solids (°Brix) during refrigerated storage

Juice combination	0 Days	15 Days	30 Days	45 Days	60 Days
100% wheatgrass juice (T ₀)	5.30	5.40	5.49	5.61	5.71
40% wheatgrass juice and 60% carrot juice (T ₃)	7.01	7.11	7.21	7.31	7.43
100% carrot juice (T ₅)	8.10	8.20	8.31	8.48	8.59
S.Ed			0.1387		
C.D at 5%			0.276		

Table E 4: Variation in vitamin-c (mg/100ml) during refrigerated storage

Juice combination	0 Days	15Days	30Days	45Days	60 Days
100% wheatgrass juice (T ₀)	25.52	25.50	24.98	24.56	24.11
40% wheatgrass juice and 60% carrot juice (T ₃)	14.17	14.14	13.81	13.08	12.84
100% carrot juice (T ₅)	4.21	4.18	4.08	3.76	3.48
S.Ed			0.0310		
C.D at 5%			0.324		

Table E 5: Variation in β -carotene during refrigerated storage

Juice combination	0 Days	15 Days	30Days	45 Days	60 Days
100% wheatgrass juice (T ₀)	6.27	6.21	6.01	5.88	5.68
40% wheatgrass juice and 60% carrot juice (T ₃)	2.30	2.20	2.11	2.01	1.78
100% carrot juice (T ₅)	8.33	8.03	8.01	7.79	7.04
S.Ed			0.0277		
C.D at 5%			0.454		

Table E 6: Variation in viscosity (cP) during refrigerated storage

Juice combination	0 Days	15Days	30Days	45Days	60 Days
100% wheatgrass juice (T ₀)	1.2	1.3	1.38	1.48	1.52
40% wheatgrass juice and 60% carrot juice (T ₃)	1.32	1.38	1.41	1.47	1.61
100% carrot juice (T ₅)	1.4	1.47	1.51	1.55	1.68
S.Ed			0.2624		
C.D at 5%			0.478		