Development of Low Sugar High Protein Smoothie for Weight Management

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By

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DEDICATION

This thesis is wholeheartedly dedicated to my beloved parents, whose unconditional love, endless prayers, and countless sacrifices have shaped who I am today. Their constant encouragement and faith in me have been the foundation of my determination and perseverance.

I also dedicate this work to my family, who stood by me with unwavering support, patience, and understanding. Their presence has been a source of motivation, comfort, and strength throughout this endeavor.

And to all those who believe in the value of knowledge and perseverance, may this achievement serve as a small token of gratitude.

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In the end, I would like to extend my deepest gratitude to my family members. Without their encouragement, I would not have been able to complete this endeavor.

Adeeba Saleem

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LIST OF ABBREVIATIONS

(Abbreviations not explained in the text)

T1, T2, T3, T4	Different smoothie formulations (treatments)
SD	Standard Deviation
LSD	Least Significant Difference
pH	Potential of Hydrogen (acidity/alkalinity measure)
TA	Titratable Acidity
cP	Centipoise (unit of viscosity
ANOVA	Analysis of Variance

Abstract

Purpose: The creation of low-sugar, high-protein foods with tempting sensory qualities and stability is required due to the increased interest in functional beverages for weight management. In order to maximize nutritional content, sensory acceptance and short-term storage stability, this study set out to create plant-based smoothies utilizing vegan milk substitutes.

Methods: Four formulations (T1–T4) were made using different amounts of almond milk and additional substances sourced from plants. Viscosity, pH, titratable acidity, stability and proximate composition were measured. An untrained panel assessed the color, flavor, texture, taste, and general enjoyment using their senses. ANOVA was used to evaluate the data and $p < 0.05$ was considered significant.

Results: While T4 recorded the highest moisture (85.60) and strong stability with minimum phase separation, T1 had the lowest moisture (83.10) and the highest protein in T4 (36.43). The highest levels of fiber (14.37), fat (18.95), and lowest carbohydrates (32.35) were seen in T4. The pH fluctuated between 6.45 (T4) and 6.30 (T1). T1 had the highest titratable acidity (0.099 ± 0.002 N), while T3 had the lowest (0.045 ± 0.001 N). The range of viscosity was 1600 cP (T4) to 1550 cP (T3). T4 was scored highest for taste, flavor, texture and overall acceptability in the sensory examination, whereas T4 received the highest rating for stability. The sugar amount was lowered in all formulas without sacrificing flavor.

Conclusion: With its balanced lipid profile, high protein, and high fiber content, formulation T3 was found to be the best product in terms of sensory acceptance, while formulation T4 had the highest physical stability.

Keywords: Low-sugar smoothie, high-protein beverage, plant-based milk.

Chapter1

INTRODUCTION

Obesity is a very serious and common issue these days. 21.6% of the world population is affected by obesity, and found more in developed countries. The accumulation of fat in excessive amounts in subcutaneous tissue, adipose tissue, and different organs in the body is called obesity. It is not that harmful if fat is distributed in the body in a balanced amount, but if it excessively accumulates at a single point, it can cause serious health issues. The accumulation of fat in the belly can cause obesity, cardiovascular disease, or a tumor. While it also causes major musculoskeletal diseases like osteoarthritis and it also has a bad impact on mental health, and it also limits the independence of life. Obesity has severe effects on health, so it is important to do proper and timely management. To take preventive measures to manage obesity, it is very important to diagnose it first, and its diagnosis is based on an anthropometric scale i.e., BMI, stated as a body mass index tool by measuring the body fat percentage and the waist-to-hip ratio(1).

Obesity is a complex disease; it has multiple causes, and it could be genetic or acquired. According to WHO, obesity is a global pandemic; its increased prevalence affects the whole world, and its preventive measures are not sufficient and well-known, due to which quality of life has been compromised, and the death rate has also increased. Obesity can be managed by behavioral interventions and by adopting a healthy lifestyle (2).

Obesity causes serious health conditions like cardiovascular diseases, diabetes, gout, cancer, gallbladder disease, osteoarthritis, etc. It is also stigmatized in our society and causes depression and anxiety-like mental conditions (3). Obesity has shown adverse effects on human life. When the body fat increases the life expectancy decreases. It not only decreases the life expectancy but also causes severe health problems (4).

A human body contains cells, tissues, and multiple organs, made up of lipids, proteins, water, minerals, and carbohydrates and found in a balanced and constant amount in the healthy body. When this body composition is abnormal, the body

could be turned overweight or underweight and this abnormal composition is measured by body mass index (5).

BMI is a valid tool for the measurement of body weight in order to evaluate if the person is carrying a low weight or is overweight. So, it is classified into underweight, normal weight, overweight, and obesity. The body mass index of a person between 26 to 30 is considered overweight, and more than 30 is obese, while a BMI less than 20 is considered underweight. On the other hand, BMI values between 20 to 25 are considered normal weight ranges (6). The changes in food preferences and lack of physical activity are becoming a reason for obesity (7). It is believed that obesity is caused by an imbalance between calorie consumption and energy released from the body(8).

According to the US Academy of Nutrition and Dietetics and the WHO, successful weight management and a healthy lifestyle can be achieved by adopting good dietary habits. Food with excess sugar and fat plays an important role in appetite regulation and obesity by stimulating the hypothalamic region in the brain, so it is crucial to change unhealthy lifestyles with conscious behavior. Experts are continuously working to develop medicines and dietary substances to control obesity, along with a healthy lifestyle approach. It is observed that intermittent fasting with a low-fat, low-carbohydrate, and high-protein diet effectively manages overweight. It also creates a balance between the energy intake and its usage. The control over obesity improves the overall health status, decreases the risk factors of co morbidities, and the mortality rate (9).

Studies have shown great interest in fruit and vegetable-made beverages and juices in recent years. The industries based on fresh vegetables and fruit juices have been growing rapidly worldwide. The awareness about fresh food benefits health and sensory properties has been spreading remarkably. Overall, these juices have shown excellent attributes, but these beverages are ready to serve and cannot be preserved for a longer time due to the presence of some microorganisms, e.g., Escherichia coli, Salmonella, etc. These microorganisms spoil the fruit and vegetable-made drinks after some time. Hence, these products can be saved by boiling or heating, but this can lessen their productivity and significance by changing their physical and chemical properties, consequently, their sensory properties also change. (10).

A smoothie is a thick and consistent beverage, like a semi-liquid made with fruit and vegetable juice or puree. It does not separate the juice and fibers of the fruit, so it is

healthier and more nutritious. Smoothies made with natural and fresh fruits and vegetables are rich with nutrients and minerals and help balance the body's demand to overcome metabolic disorders like obesity, cardiovascular disease, and diabetes (11).

The smoothies that are high in protein and low carbohydrates are the best way to keep you full and energetic in a healthier way to burn fat present in the body. The primary source of protein is dairy, poultry, eggs, fish, and beef, while the secondary source of protein for our smoothies and beverages is protein powder. These smoothies can help regulate food choices and control our hunger. A smoothie with balanced ingredients can help balance blood sugar levels, which is very important to keep the body in equilibrium and function properly. A balanced blood sugar level is not only important for the diabetic person but for everyone and this balance depends on a person's choice of diet. The most important role of a protein diet is to build the body's muscle mass and weight loss by helping the body to lose extra body fat (12).

As the fat increases, the muscle mass decreases and there is evidence that shows loss of skeletal muscle mass due to the accumulation of excessive fat in the body, and it is very common in menopausal women, athletes, people with metabolic disorders and the older population. The sedentary lifestyle and inactivity are risk factors for more fat and less muscle. To avoid the loss of muscle mass, it is necessary to do physical activity and take protein, which is a major source of muscle health, on the daily basis to keep the body healthy and strong. A daily dose of protein required by the body is 0.8 grams per kilogram of an individual's body weight, to maintain metabolic health and weight (13).

Whey proteins contain bioactive substances like amino acids and exhibit high nutritional significance. Its use is very effective in managing blood pressure and reducing obesity by lowering fat content in the body, it also shows anti-inflammatory and anti-oxidative properties. It acts as an emulsifier and adds texture to food (14). Whey protein is very nutritious as it is made up of nearly 400 substances and making it rich in nutrition and minerals, many of which are beneficial for health, but some have adverse effects on health. The great properties of whey protein help in muscle building, which is also very help full for athletes to fulfill their needs while doing heavy exercises. Whey protein is no doubt very beneficial and has great health outcomes, but it should be taken under the supervision of an expert for better results

and unsupervised or unadvised consumption can cause bad effects on one's health (15).

Apple is a very common fruit and is widely consumed all over the world. Apple has many types red-apple, golden apple, Granny Smith, Gala and many more. Each type has its own properties related to texture, taste and aroma. Apart from apples, apple juice is also very famous and a favorite to drink among people of all ages."An apple a day keeps a doctor away" is a very famous saying, which interprets the nutritional benefits of apples on human health(16). This is frequently quoted because of the extraordinary properties of apples. Apples are full of nutrition and minerals, considered as a highly functional food. It mainly contains zinc, iron, and vitamins C and E. The daily intake of apples reduces the chance of several chronic metabolic diseases like cancer, obesity, diabetes, and cardiovascular diseases. It also reduces oxidative stress, gastrointestinal tract problems, inflammation, and overall health (17).

Recently, chia seeds have come into the spotlight due to their magnificent properties. It is not only used as a dietary product, but it also has many therapeutic effects. It contains omega-3 fatty acids and protein, and with its therapeutic use, it can reduce inflammation and serve as an antioxidant. The chemical properties of chia seeds help reduce obesity and high cholesterol. Some research showed that using 40 grams of chia seeds daily for 12 consecutive weeks reduces hypertension and diabetes because it is rich in bioactive substances like phytosterols, polysaccharides, and phenolic compounds (18).

Fruit and vegetable smoothies are rich sources of energy and fiber. Recent studies showed that chia seeds are nutritious and full of fiber; 100 grams of chia seeds fulfill the daily requirement of fiber intake of an adult because they contain almost 36-40 grams of fiber. Chia seeds are safe to use and have no side effects; they lower the level of blood glucose, cholesterol, and high blood pressure (11). To decrease weight, a daily dose of 40 grams of chia seeds is sufficient to maintain a healthy body(19).

Another high-energy food is almond, which consists of iron, folate, magnesium, calcium, proteins, fiber, vitamin E, niacin, saturated fats, and carbohydrates, but are low in energy density, and it has sufficient nutrients to fulfill one's needs. Unlike other nuts, almonds, are taken into the diet, can reduce body fat and weight as it is a good weight controller. Almonds have a unique property to reduce hunger and

maintain the body's energy level by increasing and using resting energy. A daily dose of 42g of almonds is enough to improve health by lowering the risk of coronary heart disease, cardiovascular disease, high blood pressure, skin aging, and mental stress. It also improves cognitive skills and a person's overall performance (20).

Almonds are rich in vitamins, minerals, good fats, and fiber, it is very effective in maintaining metabolic health. They also contain some unique characteristics. it is observed that not only the inner part of the almond is beneficial, but the outer skin is also very important and serves as an antioxidant (21).Almond milk is also a very good alternative to cow milk. It is observed that cow milk is rich in fats and has less calcium, but in contrast to cow milk, unsweetened almond milk is full of nutrients and contains half the percentage of fats present in cow milk, and it also contains more calcium than the milk contains from other sources (22).

Greenleaf vegetables like spinach are also full of nutrients and minerals such as vitamins C, K, and A, folate, iron, magnesium, and polyphenols. It is characterized as a good anti-inflammatory and anti-oxidant product and can decrease the chance of many chronic diseases, like oxidative stress, breast cancer, prostate cancer, gallstones, and intrahepatic stones. It is an inexpensive way to improve diet quality. Its regular intake also decreases the risk of developing non-alcoholic fatty liver disease (23). Due to its therapeutic and dietary benefits, dietitians and health workers recommend its daily and regular consumption. Spinach is a very low-calorie but healthy food and is perfect for dealing with malnutrition and starvation. It also prevents the spread of non-communicable diseases like obesity (24). For a long time, spinach has been used as a food source for animals, but as the population and awareness grow, spinach has become a highly demanding and widely consumed dietary product and can be cultivated all over the world because it is an environmentally friendly product. The dried form of spinach contains 30-35% of protein, it a good amount to fulfill a human need. By evaluating its nutritional benefits, a daily dose of 170 grams of spinach is safe for a healthy body and easily digestible (25).

In recent years, obesity has grown rapidly and is mainly due to the change in food preferences, like the consumption of high-calorie and processed food rich with fats, carbohydrates and sugar. This high-calorie food leads to the development of non-communicable diseases in almost 36 million people in the world. To overcome this pandemic, several preventive measures have been taken at the state level and

privately at the population level, by altering food preferences, active lifestyle and behavior changes. In this regard, low-calorie food items are encouraged to consume and kale is one of the foods that has all the micronutrients required for a healthy body. It looks like cabbage and is mostly used in its raw form as a salad. It is stated as a super dietary item and contains vitamins, minerals, polyphenols and antioxidants (26). Kale is also a green food found in Central Asia. It has many health-promoting benefits and acts as a treatment for many chronic diseases, such as cardiovascular diseases and gastrointestinal tract disorders. It can be significantly used as an anti-carcinogenic and antioxidant (27).

Rationale of Study

The thorough study of existing literature showed an extreme need to develop a quick, easy, and nutritious smoothie recipe to manage and improve increased weight problems in young adults. A smoothie that contains low sugar but high protein to fulfill the young generation's calorie needs, so they cannot feel energy deficient with a single serving of a freshly made smoothie and perform every task effectively without getting worried about increased weight management problems.

AIM AND OBJECTIVE

- To develop an innovative low-sugar and high-protein smoothie to manage weight in individuals.

Chapter2

LITERATURE REVIEW

The increasing prevalence of obesity and overweight conditions has encouraged the development of functional foods that promote healthy weight management. Previous studies have focused on the role of high-protein and low-sugar diets in improving metabolism and reducing body fat. Functional ingredients such as whey protein, chia seeds, almonds, and plant-based milk have shown significant health benefits in this regard. Therefore, reviewing past research is essential to identify the nutritional and functional components that can be utilized in developing a low-sugar, high-protein smoothie for effective weight management.

Lui et at (28) conducted a study to evaluate the impact of intermittent meal replacement, a protein-rich diet with physical training, to reduce weight in obese females aged 18 to 35 years. A total of 40 participants took part in this randomized controlled trial study with the sealed envelope randomization sampling technique. This study was completed in 8 weeks. The inclusion criteria were that body fat must be more than 28%, BMI was 24 to 28 kg/m²and the hip to waist ratio must be more than 0.8. The exclusion criteria were dependent to diabetes, hyper/hypothyroidism, gut or stomach problems, allergy to food, hypertensive disorder, smoking, any medication, or drug addiction. Before recruitment, written consent was taken from all the participants. One group was assigned to have a protein diet with physical training and the other group had their normal routine diet with physical training. The daily dose of diet was 1000 to 1500 kilocalories. The screening included a urine sample, hematological tests and BMI, before the study, at the 3rd week of the study and after the study to evaluate the difference in both groups. A trained physical therapist was assigned to implement the exercise plan 40 minutes and maintain heart rate. The group with the protein replacement diet and physical exercises significantly reduced body weight, fasting sugar, and fat percentage. Hence, the results suggested that the intermittent meal replacement protein diet with physical training had significant effects ($p < 0.001$) on overall health, weight loss, metabolic system and fat composition rather than simple diet with physical exercises.

Rehal J et al presented a study on the challenges and benefits of milk extracted from plant sources (29). Recently, milk alternatives have become the greatest choice among the population as a healthier alternative to dairy milk. Vegan milk has been extracted from different sources like nuts, grains, and pseudo cereals. All these milk alternatives have their unique properties and characteristics, as they are rich in nutrients, minerals, and bioactive substances. Vegan milk has many health benefits, including anti-inflammatory properties, cholesterol maintenance, and good systemic health. This vegan milk is also beneficial for individuals who have any allergy to dairy milk or are lactose intolerant. Vegan milk needs fewer resources like land and water, but it still faces challenges to its wide acceptance. On the broader aspects, milk alternatives are deficient in calcium, vitamin B12 and vitamin D, which are present in dairy milk and are necessary for the human body. Its mouth feel, taste and texture are also different from the dairy milk. This study has provided a detailed analysis of vegan milk; however, more studies are required to evaluate its sensory and functional attributes.

Jahan S. et al conducted a study on the benefits of almonds on men and women in improving blood pressure, glucose and obesity (30). Almonds' consumption has shown positive effects on health in managing cardiovascular diseases and maintaining good metabolic and systemic health because they are rich in nutrients, including vitamins, fibers, good fats and minerals. It was a randomized controlled trial study. There was a total of 72 people of 35 to 55 years, participated in the study. The treatment group took 30 grams of almonds daily for 12 weeks. All the participants were analyzed at baseline and after treatment with HbA1c, FSG, blood pressure, and waist circumference. The recorded data was then statistically analyzed by SPSS, a t-test was applied and the p-value was recorded as significantly positive at less than 0.05. The results showed a 5.9% reduction in waist circumference and improvement in blood sugar with long-term usage. There was no significant change in overall blood pressure. Hence, almonds proved an excellent dietary substance for maintaining good health status.

A randomized clinical trial study was conducted in 2024 at Kobe University Hospital by Takahashi M. et al. (31). The main aim of this study was to evaluate the effects of whey protein, placebo and soy protein on obese patients. It was a double-

blinded experimental study. The inclusion criteria were BMI more than 30 kg/m^2, age more than 18 years, diabetes and stable for exercise, while exclusion criteria included any allergy with soy or whey protein, chronic kidney disease, chronic liver disease, weight instability, any obesity related recent treatment, breastfeeding, pregnancy, pacemaker and chronic thyroid disease. A total of 44 participants underwent whey protein, soy and placebo treatment along with exercises and a calculated diet according to BMI, once a day for 12 consecutive weeks. The food frequency questionnaire was used to observe the energy intake. The researcher assessed the difference between pre- and post-treatment effects on obesity. The results showed no significant change in body weight, waist circumference, blood pressure and other metabolic changes. While lipid and protein intake were found less in the soy protein group.

Lutfiye et al (32) presented a research study aimed at making a quick and easy kefir-based smoothie made up of green food, spinach, and kale. This study evaluated all the pre- and post-gastrointestinal activity, including physicochemical and microbiological components. The kefir smoothie with kale showed a high value of fructose and glucose; on the other hand, the spinach kefir smoothie showed high maltose and sucrose. The in vitro gastric and intestinal approaches showed that bio accessibility of the kefir smoothie was higher than the smoothie with vegetables. The Diphenyl-1-picrylhydrazylDPPH intestinal values were greater for the spinach smoothie than others. The results showed a deep and thorough study of all the smoothies.

A study was presented to observe and compare the effects of saturated and polyunsaturated fat smoothies in young individuals, by Stephani et al. (33). Saturated fat smoothies increase glucose levels while polyunsaturated fats raise omega-3 fatty acids. 15 individuals willingly participated in this study. They visited the research site 3 times and asked to take the special ingredient-made smoothies separately. The pre and post-effects of smoothies were measured by calculating triglycerides, metabolic load index, and glucose level, before drinking the smoothie and 2 and 4 hours after its consumption. The outcomes showed no significant difference in the SF and O3FA groups, as both triglycerides and glucose rose almost at the same level, while the metabolic load index rose in the SF group after 2 hours of smoothie

consumption, then in the O3FA group. Hence, saturated fatty acids raise the metabolic load of individuals.

Folorunso et al. (34) conducted a study to evaluate the sensory properties and nutritional properties of different fruits and vegetables mixed smoothies. Pineapple, leaves of fluted pumpkin, beetroot, and sour soup were added in proportion to make the smoothies. The first smoothie contained the highest proportion of fluted pumpkin leaves, which was 70%, while 10% was pineapple and 20% was beetroot. The ingredients of the second smoothie were 20% pineapple, 20% beetroot, and 60% of fluted pumpkin leaves. After blending and mixing, the nutritional examination and sensory evaluation were done with different means. The results showed both smoothies were enriched with fibers, proteins, and moisture, while the first smoothie contained more iron and magnesium; on the other hand, the 2nd smoothie contained more zinc. There were no fat traces found in these drinks. There was another smoothie made up of beetroot and sour soup, which had a higher potassium level. Consequently, all the smoothies were good and can be replaced with carbonated drinks because of their nutritional significance.

Dyeing et al.(35) performed a clinical trial experimental study on 139 obese individuals who had to follow a caloric restriction diet with a time restriction and a calorie restriction diet without time restriction. After following the inclusion criteria and randomization step, each group contains 69 members. Only 118 members followed the strict diet protocol for 12 months. The outcomes were measured with the two-tailed test by using SAS software. The significance level was 0.025. The differences examined were changes in pre- and post-diet body weight measured by BMI, waist circumference, fat reduction in the body, and ultimately, the risk of co morbidities also decreased. The group that followed the time-restricted diet lost a little more weight than the group that only followed the calorie-restricted diet. Hence, the time-restricted diet did not show strong results, so it was not more significant and beneficial than others.

A De Cassia et al presented the effects of chia seeds on obesity, diabetes and hypertension patients (36). 59 participants took part and were divided into two groups, group A contains 34 participants with metabolic diseases mentioned above

and group B contains 25 healthy participants. The weight was measured by BMI, including waist and abdominal circumference, while antioxidant status was measured by the MTT reduction tool. Chia seeds were given to both groups for 6 months. The pre- and post-consumption of chia seeds showed significant outcomes with a p-value less than 0.05 for both groups. Both the group members benefited from weight reduction and the antioxidant levels in plasma increased. Consequently, chia seeds proved best in all means for both healthy persons and patients suffering from chronic diseases.

Glynn et al. (37) conducted a clinical trial study. This literature was based on the consumption of a high-protein diet and fiber vs a low-protein and fiber diet in the form of shakes to improve weight and overall health. A total of 206 obese and overweight participants were randomly allocated to both groups. They were instructed to take shakes before a half-hour of a limited-calorie breakfast and lunch, for 12 consecutive weeks. Both groups undergo specific criteria to examine the results. After statistical analysis, both groups showed weight loss as a primary outcome, while the group that took high-protein and fiber shakes showed greater results for weight loss than the second group. While metabolic changes were also seen in both groups, but were greater in the first group. LDL, oxidized and serum total cholesterol levels also decreased while the level of adiponectin was raised. This literature concludes that a specific ingredient diet, including high proteins, bioactive compounds, and fiber content, has significant benefits on health as well as weight.

Alfawaz et al conducted a cross-sectional study on the benefits of kale, its knowledge, and awareness among people of the Kingdom of Saudi Arabia (26). An online survey was conducted on 1200 adults based on a questionnaire about the socioeconomic properties of kale, its awareness and knowledge. The participants were asked if they had ever tried kale or observed any health benefits after its consumption. Only 23% of people have tried kale out of which 17.8% reported ad effects like constipation and digestion issues, while 64.5% reported excellent results like weight loss. The 2nd second part of the survey contains knowledge questions and was scored from 0-10. The average knowledge of females was 3.7 and males was 3.5. The demographic data showed that the person with higher education and income was more aware of kale's health benefits than the participants with lower income and

less education. The results showed less knowledge and awareness about this nutritious food. Hence, there should be more awareness all over Saudi Arabia, especially among young adults with low income and less education.

Batsis, J. A., et al. conducted a study aimed at evaluating the effect of whey protein in reducing weight in obese adults (38). It was a non-randomized study. A total of 28 participants took part in this study for 12 weeks. All the participants were divided into two groups; one group had a protein diet and the other had a non-protein diet thrice a day, along with physiotherapy strength training exercises. The physiotherapist and dietitian and nutritionist visited two times in a week to observe the changes. The strength training evaluation tests included a grip strength test, a six-minute walk test and a sit-to-stand test. The difference in the body's composition was observed with a self-report that included overall physical health and function-related questions. The measured differences were then statistically analyzed on SPSS. The results suggested that there was significant weight loss in both groups, including the group with the protein diet and without the protein diet. There was no difference in the 6-minute walk test and sit-to-stand test in both groups, while the strength of grip was increased in the group that had a protein diet. Moreover, whey protein had a good taste, easy availability and affordability. Hence, consequently, whey protein had a positive effect in reducing weight along with physical training in older obese adults.

Vellee et al. conducted a study on the benefits of the daily intake of apple juice on human health (39). Many researchers investigated the properties of apples, as it is easily accessible, cheap, multi-nutrient, and versatile. Apple is a rich source of bioactive substances, and its juice is a super source to consume all the necessary bioactive agents. Apples are consumed in many different ways, such as raw apples, juice, cider, pomace, and vinegar. The cloudy apple juice offers more benefits and contains the maximum nutrients, fulfilling the body's daily need for fruit consumption. These studies revealed that apple juice had a great effect on cardiovascular health, neurodegenerative disorders, and cancer, while it was also effective for metabolic diseases like diabetes and obesity. Hence, apple juice has positive effects on overall health and reduces obesity related risk factors and other health conditions.

Jan et al (9) conducted a systematic review aimed to evaluate the health status and management of weight loss with the help of dietary habits and drugs. Different search engines were used to evaluate the authentic data published after the year 2005. All the studies relevant to the low-carbohydrate, low-fat, intermittent fasting diet and the Mediterranean diet were included. The underlying purpose of this weight management was to maintain an energy equilibrium and long-term adherence to diet in the body. A low-carb diet seemed beneficial for type 2 diabetes patients who were on GLP1 agonist drugs. The individuals with hypercholesterolemia using concerned medications benefit from a low-fat diet. The results showed that appropriate diet choices improve overall health status and a healthy weight reduces the risk of developing obesity-related comorbidities. Consequently, clinicians must advise a healthy lifestyle while prescribing concerned medicines for the disease.

Abdul Basit et al (40) surveyed to evaluate the prevalence of obesity in Pakistan. This literature was analyzed by a National diabetic survey held in 2016 to 2017 in Pakistan. A total of 10,834 individuals from all 4 provinces took part in this study. A multistage sampling technique was used in this study. All the participants were fasting the night before the survey. After following all the parameters of inclusion and exclusion criteria, OGTT, weight, height, and circumference of the waist were determined. The collected data was then analyzed on SPSS. The results showed that the prevalence of general obesity and central obesity was 57.9% and 73.1%, respectively.60% of generalized obesity was found in Punjab and 59.2% was found in KPK. While 82.1% central obesity was found in Baluchistan and 73.3% found in Punjab. The conclusion showed that obesity is an epidemic condition in Pakistan and there is an urgent need to intervene in protocols to overcome and further prevent obesity and its related co morbidities.

Basu A. et al conducted a randomized controlled trial study on the effects of dietary fibers and blueberries on obese women had risk of gestational diabetes (41). There were 34 women with less than 30 weeks of pregnancy recruited and allocated to the two different groups, one was the treatment group and the other was the control group. The treatment group took blueberries and soluble dietary fibers and the

control group had standard care only. The daily dose of blueberries was 280g and dietary fiber was 12 g for 18 weeks. The pre- and post-treatment changes were observed by blood pressure evaluation, anthropometric data, and blood samples at baseline, at the mid of treatment and the end of treatment. While gestational diabetes was evaluated with GCT. The collected data was then statistically analyzed by SPSS by applying the ANOVA test. The results showed a significant decrease in the weight, C-reactive protein, and blood glucose in the treatment group members than the other group members. Hence, it was proved that blueberries and soluble dietary fibers were beneficial in controlling excess weight, inflammation and glucose in obese women.

Land Lail, H. et al. presented a study on the benefits of berries for obese people in improving inflammation associated with obesity (42). Obesity is a common problem among the adult population and it is growing rapidly all over the world and it might increase by up to 48% in 2030 in the United States. Obesity has many adverse effects on the liver, pancreas, adipose tissues, brain and heart. Obesity control is considered a key factor in overcoming and controlling many chronic systemic diseases like cardiovascular diseases, diabetes, cancer, etc. Healthy food consumption has good effects in improving obesity. Berries are considered one of the best food choices, as studies have shown their anti-inflammatory effects on the human body. Berries are a multi-nutrient product; they contain fibers, vitamins, bioactive substances, minerals, and polyphenols. It has been widely consumed all over the world due to its anti-inflammatory effects on health. There is a variety of barriers available, i.e., strawberries, blueberries, raspberries and blackberries. Hence, this study showed the effects of berry consumption in reducing diabetes, cardiovascular diseases, and inflammation associated with obesity etc.

Stull, A. J. etal, conducted a study on the bioactive present in blueberries and its impact on improving insulin sensitivity in non-diabetic obese males and females (43). A randomized controlled trial study was used to perform this experiment. It was a double-blinded study. Thirty-two participants, including both males and females, took part in this study. The body mass index of all the adults ranged between $32kg/m^2$to $42kg/m^2$. The inclusion criteria were specific to individuals with insulin resistance aged 20 years or older. While the exclusion criteria were people

having an allergy to blueberries, drug or alcohol addicts, any untreated systemic diseases like CVS, kidney or liver disease, and thyroid diseases, diabetic patients. Informed written consent was taken from all the members before starting the study. All the members were fasting for ten hours before taking the smoothie. One group was given a blueberry bioactive smoothie while the other group was given a simple smoothie with blueberry bio-actives for 6 weeks, 2 times a day. After 6 weeks, insulin sensitivity, body weight, energy intake and inflammation were assessed again and only insulin sensitivity of the group having blueberry smoothies improved; all the other biomarkers remained the same. Hence, blueberry daily consumption improves insulin sensitivity in the non-diabetic obese population

A comprehensive review of the previous literature has identified a lack of effective low-sugar, high-protein smoothies that do not cause feelings of less energy, dizziness, and fatigue, which can help manage overweight and obesity in today's busy and fast-paced era among young adults aged 20-30 years. Though both components in different studies showed beneficial and significant results when considered in the diet for weight loss and energy management. Hence, there is still a lack of research to merge these two components into a single, quick,and smoothie-like product that will be available anytime without any time restrictions and fulfill the need for energy with low calories.

Chapter3

METHODOLOGY

3.1. Study Design:

It was a product development study

3.2. Duration of Study

The duration of study was 04 months.

3.3. Place of work

Smoothies were prepared in the culinary lab of Superior University, Lahore

3.4. Product Development

3.4.1. Materials

The materials required for this study were easily available in the local markets of Lahore. The ingredients included almonds, chia seeds, kale, spinach, apples, blueberries, whey protein, water, and vanilla extract. All these materials were procured fresh and of high quality to ensure the nutritional integrity and consistency of the smoothie formulations.

3.4.2. Preparation Methods

Preparation of Almond Milk

The procedure for making unsweetened almond milk involved 150 grams of shelled almonds, 400 milliliters of water, and 5 milliliters of vanilla extract. First, the almonds were placed in a bowl and 400 ml of water was added to soak them well for 8-12 hours overnight. After soaking, the excess water was drained, and the almonds were peeled. Next, the peeled almonds, 1000 milliliters of water, and 5 milliliters of vanilla extract were added to a high-speed blender. The ingredients were then blended for 1-2 minutes until a smooth texture had been achieved. Then, the mixture was poured through a fine mesh strainer to extract the almond milk. Finally, the

prepared milk was stored in an airtight jar or bottle and placed in a cool place. It can be stored in a refrigerator for 4-5 days, but shake well every time before use.

Preparation of a smoothie

To develop the smoothie, unsweetened almond milk was taken as shown in Table 3.5, 14 grams of soaked chia seeds, 30 grams of spinach, 100 grams of kale, 250 grams of chopped apples, 280 grams of chopped blueberries, 30 grams of whey protein powder, and ice (as needed), the ingredients were weighed accurately, as shown in Table 3.5.

The first step was to soak the chia seeds in a water-filled bowl for 1-2 hours before preparing the smoothie. Then, the fresh fruits and vegetables were rinsed and cleaned, chopped separately and set aside. The smoothie was prepared using a clean high-speed juicer blender. All the ingredients were added to the blender and mixed well until a thick and smooth texture was obtained. After blending for 2-3 minutes, the quick and easy smoothie was ready to serve. The next step was to perform sensory, physical, nutritional, and chemical examinations on the smoothie.

3.5. Treatment Plan

Treatment	Almond milk %
T1	0%
T2	2%
T3	4%
T4	6%

3.6. Product Analysis

- **Physiochemical Testing:**

3.6.1 Protein Content

The protein content of the developed smoothie formulations was determined using the Kjeldahl method as described by AOAC (44). This method involves the digestion of the sample in concentrated sulfuric acid using a catalyst to convert organic nitrogen into ammonium sulfate. The mixture is then distilled after neutralization with sodium hydroxide, and the liberated ammonia is collected in a boric acid

solution and titrated with standard hydrochloric acid. The total nitrogen obtained was multiplied by a conversion factor of 6.25 to estimate the total protein content.

$$\text{Protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

This analysis determines the total protein available, which plays a vital role in enhancing satiety and supporting weight management.

3.6.2 Moisture Content

Moisture content was determined by oven-drying the samples at 102°C for 24 hours in a hot air oven, following the procedure outlined in AOAC (44). The loss in weight of the sample during drying represented the moisture content. This analysis is essential to evaluate the shelf stability and texture of the smoothie formulations, as higher moisture content can influence microbial growth and product consistency.

$$\text{Moisture (\%)} = \frac{W1-W2}{W1} \times 100$$

Where W1 = Weight of sample before drying W2 = Weight of sample after drying Moisture determination helps assess the storage stability and microbial susceptibility of the developed smoothie.

3.6.3 Fat Content

The fat content was estimated using the Soxhlet extraction method as per AOAC (45). Approximately 5 grams of the dried smoothie sample were wrapped in filter paper and placed in a Soxhlet apparatus. Petroleum ether was used as the extraction solvent for 6–8 hours until the complete extraction of lipids was achieved. After evaporation of the solvent, the remaining fat was weighed to calculate the total fat percentage. This analysis helps determine the energy contribution and nutritional quality of the formulated smoothies.

$$\text{Fat (\%)} = \frac{W2-W1}{Ws} \times 100$$

Where W1= Weight of empty flask W2 = Weight of flask + extracted fat, Ws = Weight of sample

Fat content indicates the energy contribution and mouth feel of the smoothie.

3.6.4 Carbohydrate Content

Total carbohydrates were calculated indirectly using the difference method as described by AOAC (46). The percentage of carbohydrates was obtained by subtracting the sum of protein, fat, moisture, and ash contents from 100.

$$\text{Carbohydrates (\%)} = 100 - (\text{Protein} + \text{Fat} + \text{Moisture} + \text{Ash}$$

This estimation is significant to ensure that the developed smoothie remains low in sugar and suitable for weight management.

3.6.5 Ash Content

The ash content was determined by incinerating the sample in a muffle furnace at 550 °C until a constant weight was achieved, according to the method described by AOAC (47). The residue left after complete combustion represents the total mineral content of the smoothie. Ash determination provides information about the presence of essential minerals such as calcium, potassium, magnesium, and iron, which contribute to the nutritional value of the formulation.

$$\text{Ash (\%)} = \frac{W2 - W1}{Ws} \times 100$$

Where W1 = Weight of empty crucible, W2 = Weight of crucible + ash, Ws = Weight of sample

The ash content indicates the total mineral composition of the smoothie.

3.6.6 pH Measurement

The pH of each smoothie formulation was measured using a digital pH meter standardized with buffer solutions of pH 4.0 and 7.0, as described in AOAC (44). Approximately 10 mL of the smoothie sample was homogenized and measured at room temperature. pH determination is crucial for assessing the product's acidity, flavor balance, and stability during storage.

3.6.7 Viscosity

The viscosity of the formulated smoothies was determined using a Brookfield viscometer at 25 °C, following AOAC (44) guidelines. The readings were taken at a constant spindle speed to evaluate the flow behavior and consistency of the samples. Viscosity plays a vital role in determining the texture, mouth feel, and consumer acceptability of the final product.

$$\eta = K \times T$$

Where η= Viscosity (cP), K= Instrument constant, T= Torque reading Viscosity determines the texture, mouth feel, and consumer acceptability of the formulated smoothie.

3.6.8 Stability Test

The stability of the smoothie formulations was assessed by storing the samples in glass containers at refrigeration temperature (4°C) for 24 hours and observing for phase separation, sedimentation, or coagulation, as per AOAC (44). The physical appearance and homogeneity were recorded to evaluate the emulsion stability and shelf-life potential of the product.

3.6.9 Titratable Acidity

Titratable acidity was measured according to the AOAC method (48) by titrating 10 mL of the smoothie sample against 0.1N NaOH using phenolphthalein as an indicator. The acidity was expressed as a percentage of lactic acid equivalent. This parameter indicates the degree of fermentation, product freshness, and flavor stability of the smoothie.

3.7. Sensory Evaluation

Recruited 30 untrained panelists for sensory evaluation. The samples were coded and served to 20 untrained panelists. The panelists were asked to indicate their preferences using a 9-point Hedonic scale for appearance, mouth feel, taste, aftertaste, aroma, and overall acceptability (47).

3.8. Data Analysis

Statistical Analysis:

By using SPSS version 25.0 software, data of different parameters, including sensory scores, were analyzed by finding mean, standard deviation and standard error. When examining the impact of a single factor (such as protein levels) on dependent variables, use a one-way ANOVA(44).

Chapter 4

RESULTS

4.1 MOISTURE CONTENT

The Table 4.1 presents the moisture content of low-sugar, high-protein smoothies formulated with different ingredient combinations. The moisture content increased gradually from T1 to T4. The lowest moisture value was observed in T1 (85.60%), while the highest was recorded in T4 (83.10%). Treatments T2 (84.75%) and T3 (83.90%) showed intermediate values that were statistically similar to each other but significantly higher than T1 and lower than T4. Treatments assigned the same letters indicate non-significant differences, whereas those with different letters represent statistically significant variation in moisture content. The increase in moisture content from T1 to T4 could be attributed to the varying proportions of protein sources and liquid components, which influence water-binding capacity in the smoothies.

Table 4.1. Comparison of means for moisture content of low-sugar, high-protein smoothies

Treatment	**Moisture (%)**
T1	85.60 ± 0.13d
T2	84.75 ± 0.23c
T3	83.90 ± 0.21b
T4	83.10 ± 0.29a

Values: Mean ± SD; different superscript letters indicate significant difference ($p < 0.05$).

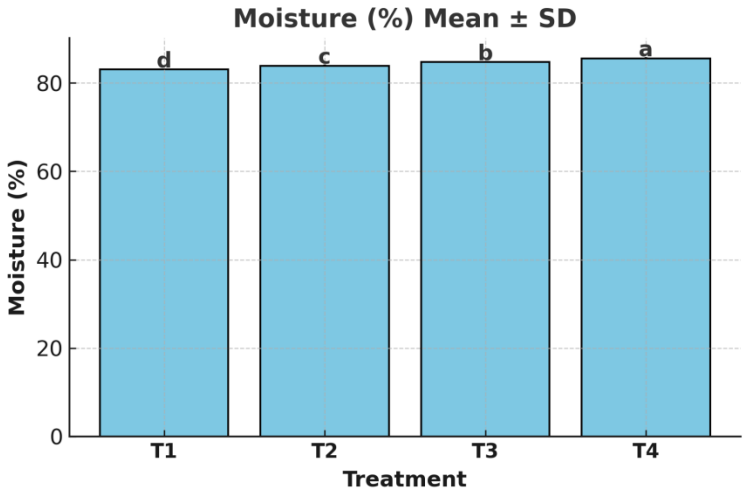

Figure 4.1. Graphical representation of moisture content variations across smoothie formulations.

4.2 Protein Content

The results in Table 4.2 show that the protein content of the low-sugar, high-protein smoothies varied significantly among the treatments. The protein content ranged from 27.80% to 30.10%, with T4 exhibiting the highest protein percentage (30.10%), followed by T3 (29.35%) and T2 (28.90%), which were statistically similar as indicated by the same letter grouping ("b"). The lowest protein content was observed in T1 (27.80%).The differences may reflect the varying ratios of protein-rich ingredients and other constituents that can dilute protein concentration.

Table 4.2. Comparison of means for protein content of low-sugar, high-protein smoothies

Treatment	Protein (%)
T1	27.80 ± 0.18c
T2	28.90 ± 0.20b
T3	29.35 ± 0.22b
T4	30.10 ± 0.24c

Values: Mean ± SD; different superscript letters indicate significant difference ($p < 0.05$).

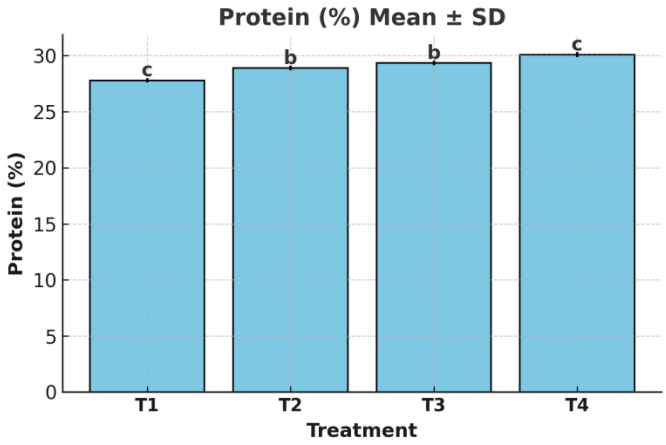

Figure 4.2. Graphical representation of protein content variations across smoothie formulations.

4.3 Fiber Content

The Table 4.3 presents the fiber content of low-sugar, high-protein smoothies prepared using different formulations. The results showed a gradual increase in fiber content from T1 to T4. The highest fiber values were recorded in T3 (13.45%) and T4 (14.37%), which were statistically similar to each other, as indicated by the same superscript letter ("a"). T1 (11.82%) and T2 (12.87%) showed lower fiber contents and shared the same letter grouping ("b"), demonstrating no significant difference between them.

Table 4.3. Comparison of means for fiber content of low-sugar, high-protein smoothies

Treatment	**Fiber (%)**
T1	11.82 ± 0.30b
T2	12.87 ± 0.32b
T3	13.45 ± 0.34a
T4	14.37 ± 0.36a

Values: Mean ± SD; different superscript letters indicate significant difference ($p < 0.05$).

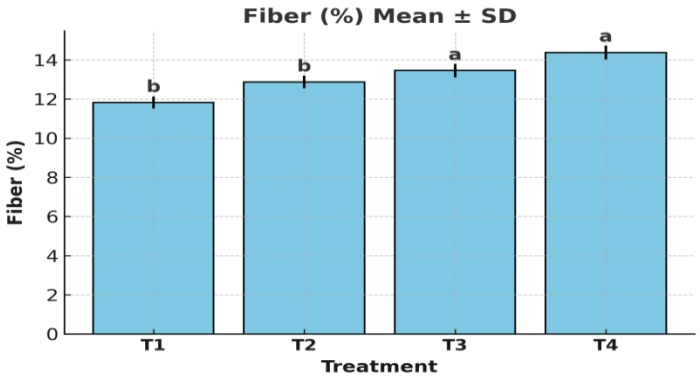

Figure 4.3. Graphical representation of fiber content variations across smoothie formulations.

4.4 Fat Content

As shown in Table 4.4, the fat content of low-sugar, high-protein smoothies varied significantly among the treatments. The highest fat value was observed in T4 (18.95%), followed by T3 (17.42%) and T2 (16.14%), while T1 (14.09%) recorded the lowest fat content. The letter groupings indicate that all treatments were significantly different from each other ($p < 0.05$). The progressive increase in fat content from T1 to T4 may be attributed to the increasing inclusion of almond milk and chia seeds, both of which are rich in healthy unsaturated fats. The variations in fat content reflect the influence of formulation composition, particularly the balance between plant-based fat sources and other macronutrients.

Table4.4. Comparison of means for fat content of low-sugar, high-protein smoothies

Treatment	Fat (%)
T1	14.09 ± 0.45d
T2	16.14 ± 0.48b
T3	17.42 ± 0.50a
T4	18.95 ± 0.55c

Values: Mean ± SD; different superscript letters indicate significant difference ($p < 0.05$).

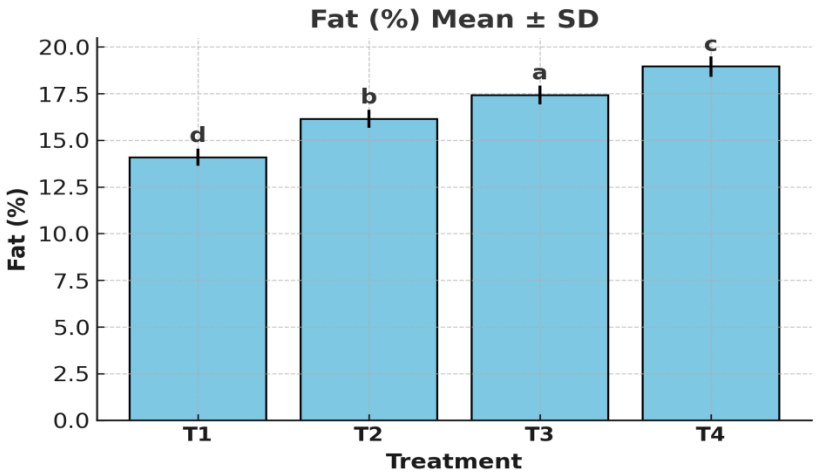

Figure 4.4.2 Graphical representation of fat content variations across smoothie formulations.

4.5 Ash Content

The Table 4.5 presents the ash content of low-sugar, high-protein smoothies prepared with different formulations. The results showed a gradual increase in ash content from T1 to T4. The lowest ash value was recorded in T1 (3.63%), while the highest was observed in T4 (4.23%). T2 (3.88%) and T3 (4.04%) exhibited intermediate values, with a statistically significant difference between them. Treatments assigned different superscript letters indicate significant differences ($p < 0.05$). These results suggest that formulation modifications can effectively enhance the mineral composition and overall nutritional quality of the smoothies.

Table 4.5. Comparison of means for ash content of low-sugar, high-protein smoothies

Treatment	**Ash (%)**
T1	3.63 ± 0.07d
T2	3.88 ± 0.08b
T3	4.04 ± 0.09a
T4	4.23 ± 0.10c

Values: Mean ± SD; different superscript letters indicate significant difference ($p < 0.05$).

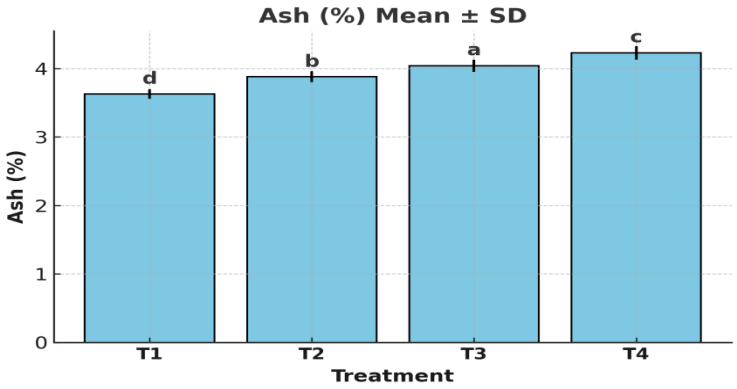

Figure 4.5. Graphical representation of ash content variations across smoothie formulations.

4.6 Carbohydrates Content

The Table 4.6 presents the carbohydrate content of low-sugar, high-protein smoothies prepared with different ingredient formulations. The results revealed significant differences among all treatments. The highest carbohydrate content was observed in T3 (19.15%), followed by T4 (15.26%), T2 (11.21%), while T1 (10.33%) showed the lowest value. Different superscript letters indicate that all treatments were significantly different from each other ($p < 0.05$). The variations in carbohydrate content could be attributed to differences in the proportions of carbohydrate-rich ingredients such as fruits and almond milk used in the formulations.

Table 4.6. Comparison of means for carbohydrate content of low-sugar, high-protein smoothies

Treatment	Carbs (%)
T1	42.66 ± 0.40a
T2	38.21 ± 0.35b
T3	35.74 ± 0.33c
T4	32.35 ± 0.30d

Values: Mean ± SD; different superscript letters indicate significant difference ($p < 0.05$).

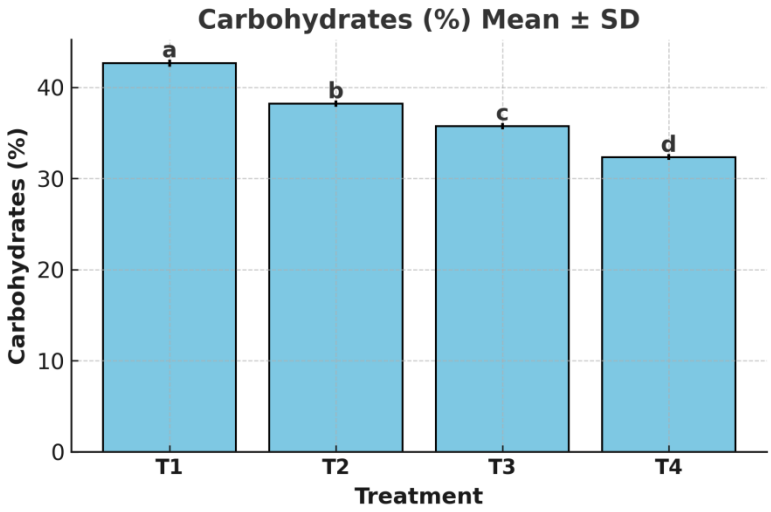

Figure 4.6. Graphical representation of carbohydrate content variations across smoothie formulations

4.7 pH Value

As shown in Table 4.7, pH values ranged from 6.30 in T1 to 6.45 in T4. T2 (6.36) and T3 (6.34) were statistically similar to T1 but significantly different from T4. The variations shows that T1, T2, and T3 share "b," indicating no significant difference among them, while T4 "a" is significantly higher. These variations may be due to ingredient acidity and buffering capacity from protein sources.

Table 4.7. Comparison of means for pH values of low-sugar, high-protein smoothies

Treatment	**pH (%)**
T1	6.30 ± 0.05b
T2	6.36 ± 0.06b
T3	6.34 ± 0.08b
T4	6.45 ± 0.07a

Values: Mean ± SD; different superscript letters indicate significant difference ($p < 0.05$).

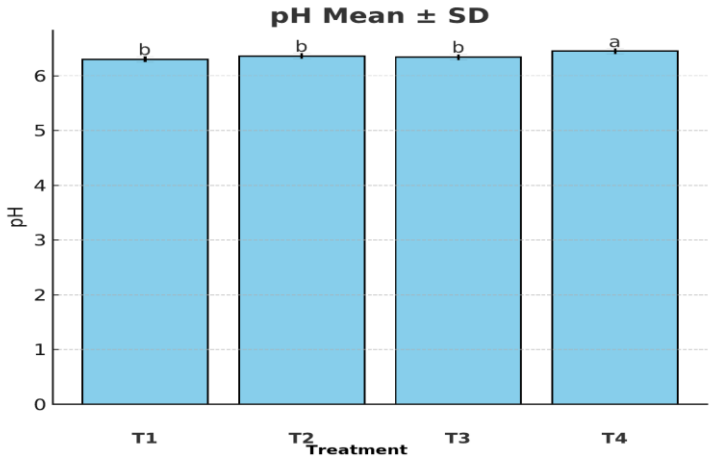

Figure 4.7. Graphical representation of pH variations across smoothie formulations.

4.8 Titratable Acidity

The Table 4.8 presents titratable acidity (TA) values. T1 recorded the highest TA (0.099N), while the lowest was found in T3 (0.045N). T4 (0.072N) and T2 (0.063N) had intermediate values, each significantly different from the others. Treatments with the same letter indicate no statistical difference; here, no two treatments share the same letter, showing all are significantly different.

Table 4.8. Comparison of means for titratable acidity of low-sugar, high-protein smoothies

Treatment	Titratable Acidity (N)
T1	0.099 ± 0.002a
T2	0.063 ± 0.001c
T3	0.045 ± 0.001d
T4	0.072 ± 0.001b

Values: Mean ± SD; different superscript letters indicate significant difference ($p < 0.05$).

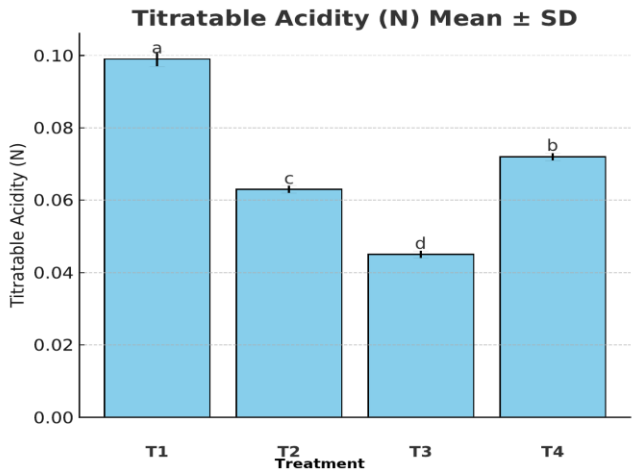

Figure 4.8. Graphical representation of titratable acidity variations across smoothie formulations.

4.9 Viscosity

The Table 4.9 presents the viscosity values of low-sugar, high-protein smoothies expressed in centipoise (cP). The results showed significant differences among treatments. The highest viscosity was recorded in T4 (1600 cP). T1 (950 cP) had a slightly lower viscosity, denoted by a different letter ("a"), while the moderate viscosity was observed in T3 (1550 cP) and T2 (1250), which differed significantly from all other treatments ("a, b, c, d"). The observed variations in viscosity can be attributed to differences in the proportions of protein, fiber, and fat-containing ingredients in the formulations

Table 4.9. Comparison of means for viscosity of low-sugar, high-protein smoothies

Treatment	**Viscosity (cP)**
T1	950 ± 10d
T2	1250 ± 15b
T3	1550 ± 20c
T4	1600 ± 25a

Values: Mean ± SD; different superscript letters indicate significant difference ($p < 0.05$).

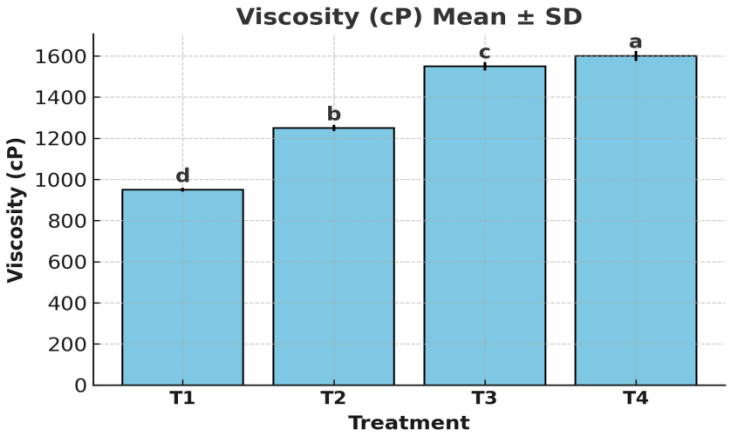

Figure 4.9. Graphical representation of viscosity variations across smoothie formulations.

4.10. Stability test for smoothie samples

The storage stability of the low-sugar, high-protein smoothies was evaluated based on appearance, smell, and layering/creaming at different storage intervals, with observations recorded for Day 0 and Day 3. These attributes provided an indication of product quality, stability, and potential spoilage during short-term storage. On Day 0, all treatments exhibited fresh and appealing visual characteristics. T1 appeared thick and vibrant, T2 had a dense and deep color, T3 was thick and smooth, and T4 displayed a uniform, creamy appearance. However, by Day 3, noticeable changes were observed in each sample. T1 showed slight dullness, likely due to pigment degradation or mild oxidation. T2 developed slight browning, which could be attributed to enzymatic browning or pH-related reactions. T3 became slightly watery, indicating partial breakdown of structural integrity, while T4 experienced slight thickening, suggesting strong stability of its colloidal system.

The smell of the samples on Day 3 further highlighted differences in stability. T1 developed a fruity-sour aroma, likely caused by the production of organic acids through microbial activity. T2's aroma became less fruity, suggesting a loss of volatile aromatic compounds. T3 had only a faint smell, which may indicate both a reduction in aromatic intensity and the onset of early spoilage. T4 maintained a balanced aroma, showing effective preservation of freshness. Layering or creaming patterns also reflected the stability of the formulations. T1 showed mild phase

separation, still within acceptable limits for short storage. T2 exhibited moderate separation, pointing to reduced emulsion stability. T3 displayed clear phase separation, a strong sign of instability and early spoilage. T4 had minimal separation, demonstrating good stability and homogeneity during storage. Overall, interpretation of the storage results indicated that T1 remained acceptable but exhibited high acidity, which may limit its shelf life. T2 was identified as pH-sensitive and required closer monitoring for stability issues. T3 showed clear signs of early spoilage and instability, making it unsuitable for extended storage. In contrast, T4 remained stable in terms of appearance, aroma, and physical integrity, making it the most promising formulation for prolonged shelf life.

Table 4.10. Changes in physical and sensory attributes of low-sugar, high-protein smoothies during storage

Sample	**Appearance (Day 0)**	**Appearance (Day 3)**	**Smell (Day 3)**	**Layering/Creaming**	**Interpretation**
T1	Thick, vibrant	Slight dullness	Fruity-sour	Mild seperation	Acceptable, high acidity
T2	Dense, deep colour	Slight browning	Less fruity	Moderate seperation	pH-sensitive, monitor
T3	Thick, smooth	Slightly watery	Faint smell	Clear phase seperation	Signs of early spoilage
T4	Uniform, creamy	Slight thickening	Balanced	Minimal seperation	Stable

4.11 Sensory evaluation

The sensory evaluation results for the low-sugar, high-protein smoothies in terms of color, taste, flavor, texture, and overall acceptability are shown in Table 4.10.1. These scores are expressed as raw means with their respective letter groupings, where identical letters denote no significant difference, and different letters indicate a statistically significant difference at $p < 0.05$. For color, the highest score was recorded for T4 **(**7.6 a**),** indicating maximum visual appeal among all treatments. T2 **(**7.2 b**)** and T3 **(**7.3 b**)** were statistically similar and moderately accepted, while T1 **(**6.9 c**)** had the lowest score, showing a visibly less appealing appearance. This suggests that formulation adjustments in T4, such as optimized fruit and protein ratios, may have enhanced its visual characteristics. Regarding taste, T4 (7.8 a) was significantly preferred over the other treatments, reflecting a more balanced sweetness and flavor depth. T2 (7.3 b) and T3 (7.4 b) were similar in taste preference, while T1 (7.0 c) received the lowest rating. The higher score for T4 could be linked to the synergy between natural sweeteners and protein components, creating a more satisfying taste profile. In terms of flavor, the results followed a similar pattern: T4 (7.7 a) ranked first, T3 (7.5 b) and T2 (7.2 b) were statistically similar, and T1 (6.9 c) was the least preferred. The enhancement in T4's flavor perception may be attributed to better integration of fruit, milk, and protein powder, contributing to a fuller and more pleasant aroma and aftertaste. The texture attribute showed T4 (7.8 a) as most preferred, indicating a smoother and more consistent mouth feel. T3 (7.5 b) and T2 (7.3 b) were again statistically similar, while T1 (7.1 c) scored the lowest, suggesting a less favorable texture. This highlights the influence of formulation viscosity and ingredient particle size on smoothie texture perception. Finally, for overall acceptability, T4 (7.8 a) stood out as the most liked formulation. T3 (7.6 b) and T2 (7.4 b) were moderately liked, and T1 (7.0 c) had the lowest overall acceptance. These results demonstrate that T4's combination of sensory qualities — high color appeal, pleasant taste, balanced flavor, and smooth texture — made it the most preferred option among panelists.

Table 4.11. Sensory evaluation scores of low-sugar, high-protein smoothies

Treatment	Color	Taste	Flavor	Texture	Overall Acceptability
T1	6.9c	7.0c	6.9c	7.1c	7.0c
T2	7.2b	7.3b	7.2b	7.3b	7.4b
T3	7.3b	7.4b	7.5b	7.5b	7.6b
T4	7.6a	7.8a	7.7a	7.8a	7.8a
LSD	0.30	0.35	0.40	0.32	0.34

Values with the same letter are not significantly different ($p < 0.05$).

The detailed sensory evaluation data, expressed as Mean ± SD, is presented in Table 4.10.2. This representation provides both the average score and the variability among panelist ratings, along with letter groupings that indicate statistical differences. As with the raw data, the same letter represents no significant difference, and different letters indicate significant differences ($p < 0.05$). For color, T4 had the highest mean score (7.63 ± 0.10 a), showing strong and consistent panelist agreement on its appealing appearance. T3 (7.28 ± 0.08 b) and T2 (7.17 ± 0.12 b) had statistically similar values, while T1 (6.94 ± 0.12 c) was significantly lower. The small standard deviation in T4 suggests a high level of agreement among panelists. In taste, T4 (7.80 ± 0.07 a) maintained its top ranking with minimal variability in scores, reflecting uniform preference. T3 (7.37 ± 0.10 b) and T2 (7.27 ± 0.11 b) were statistically similar and moderately preferred, while T1 (6.97 ± 0.16 c) was least preferred. The higher variability in T1's score indicates a less consistent perception among panelists.

The flavor results show T4 (7.71 ± 0.07 a) as the highest-rated, followed by T3 (7.50 ± 0.08 b) and T2 (7.23 ± 0.10 b) with no significant difference between them, and T1 (6.93 ± 0.10 c) lowest. The low SD values for T4 indicate panelists largely agreed on its favorable flavor. In texture, T4 (7.80 ± 0.07 a) again topped the list, with T3 (7.49 ± 0.07 b) and T2 (7.26 ± 0.09 b) statistically similar and T1 (7.07 ± 0.08 c) at the bottom. The minimal variation in T4's texture score suggests panelists consistently perceived it as smooth and pleasant. Finally, in overall acceptability, T4 (7.81 ± 0.07

a) remained the most accepted smoothie, followed by T3 (7.59 ± 0.07 b) and T2 (7.35 ± 0.11 b) as moderately preferred, and T1 (6.98 ± 0.12 c) least accepted. The consistent high means and low SD values for T4 across all parameters highlight that it not only performed best but also maintained panelist agreement.

Table 4.11.1. Sensory evaluation scores of low-sugar, high-protein smoothies

Treatment	Color	Taste	Flavor	Texture	Overall Acceptability
T1	6.94 ± 0.12c	6.97 ± 0.16c	6.93 ± 0.10c	7.07 ± 0.08c	6.98 ± 0.12c
T2	7.17 ± 0.12 b	7.27 ± 0.11b	7.23 ± 0.10b	7.26 ± 0.09b	7.35 ± 0.11b
T3	7.28 ± 0.08b	7.37 ± 0.10b	7.50 ± 0.07b	7.49 ± 0.09a	7.59 ± 0.05a
T4	7.63 ± 0.10a	7.80 ± 0.07a	7.71 ± 0.08a	7.80 ± 0.09a	7.81 ± 0.05a

Values: Mean ± SD; different superscript letters indicate significant difference ($p < 0.05$).

Chapter 5

DISCUSSION

The current research was conducted for the development of a low-sugar, high-protein smoothie for weight management. Its purpose was to make a smoothie with vegan milk by adding different percentages of almond milk, i.e., 0%, 2%, 4%, and 6%, to analyze the smoothie stability, sensory and physicochemical properties, because the concentration of milk had a visible impact on these parameters, to identify the ideal formulation. Moisture content is a crucial quality parameter in smoothie development because it directly influences texture, viscosity, mouthfeel, stability, and shelf-life Rathore et al. (49). In the present study, moisture values varied significantly among formulations, with T1 showing the lowest (7.75 ± 0.13%) and T4 the highest (16.89 ± 0.29%). These differences can largely be explained by the ratio of solids to liquids and the water-binding capacity of the protein and fiber sources used in each formulation. Protein enrichment is the central functional strategy in developing smoothies aimed at weight management, as it influences not only the nutritional profile but also satiety, body composition, and metabolic outcomes. In the present study, protein content ranged from 47.74 ± 0.22% in T1 to 43.50 ± 0.28% in T3**,** with variations likely arising from differences in protein source (whey, soy, pea, casein) and the relative proportion of other macronutrients such as carbohydrates and fats in the formulation.

Protein quality is determined by amino acid profile, digestibility, and bioavailability FAO (50). Whey protein, for example, is rich in leucine — a branched-chain amino acid (BCAA) that is a potent stimulator of the mTOR pathway, thereby promoting muscle protein synthesis and maintenance of lean mass during energy restriction Tang et al. (51). In contrast, plant proteins like pea or soy may have slightly lower leucine content but can offer functional benefits such as improved satiety due to their slower digestion rate and higher fiber content Mariotti & Gardner (52).

Clinical evidence strongly supports high-protein meal replacements in weight loss protocols. Randomized controlled trials have demonstrated that protein-enriched

shakes lead to greater reductions in body fat percentage and improved muscle retention compared to carbohydrate-based equivalents Ayyash et al. (53). This effect is amplified in low-sugar formulations, which minimize glycemic fluctuations and insulin spikes, thereby supporting better appetite control and metabolic health Ludwig (54).Therefore, a formulation like T1 high protein, low sugar, and sensorially optimized aligns closely with evidence-based recommendations for weight management, offering both nutritional efficacy and consumer compliance.

Dietary fiber, both soluble and insoluble, is a key functional ingredient in weight management smoothies due to its synergistic effects with protein in enhancing satiety, regulating postprandial glycemia, and supporting digestive health Kendall et al. (55). In the present study, T3 (8.45 ± 0.15%) and T4 (8.40 ± 0.15%) contained approximately double the fiber content of T1 and T2. This higher fiber level is significant from a nutritional standpoint, as it can substantially influence both the functional performance and consumer perception of the product. When fiber is combined with high-protein formulations, as in T3 and T4, the satiety effects can be additive or even synergistic. Protein's stimulation of anorexigenic hormones (PYY, GLP-1) combined with fiber's slowing of gastric emptying and glucose absorption can lead to a reduction in subsequent energy intake Burton-Freeman (56). This dual-action approach is particularly advantageous for weight control strategies, where sustained satiety is crucial for adherence to a hypocaloric diet. Incorporating adequate levels of both soluble and insoluble fiber into low-sugar, high-protein smoothies can enhance satiety, modulate glycemic responses, and promote gastrointestinal health — all of which are essential for effective weight management. However, careful formulation is needed to balance nutritional efficacy with sensory acceptability.

Fat plays a multifaceted role in smoothie formulation, influencing mouthfeel, flavor delivery, satiety, and nutrient absorption. In the present study, T3 exhibited the highest fat content (48.05 ± 0.30%), a level that strongly suggests inclusion of fat-rich ingredients such as nut butters, seeds, coconut products, or full-fat dairy. These ingredients not only contribute to creaminess and flavor depth but also provide essential fatty acids and fat-soluble vitamins (A, D, E, and K) that are important for overall health Calder (57). However, excessive fat content — such as that seen in T3

— can substantially increase caloric density, potentially undermining the calorie-control goals essential for weight loss. A strategic approach in smoothie formulation is to maximize the nutritional quality of fat while controlling its quantity. This can be achieved by prioritizing MUFA- and PUFA-rich plant-based sources (e.g., almond butter, flaxseed, chia seeds) over saturated fat-dense options, while also considering the emulsification properties of fats in improving texture and stability McClements (58). Such ingredient selection can help deliver the creamy mouth feel and flavor intensity consumers expect, without exceeding energy targets for weight management.

Ash content is an important indicator of the total mineral concentration in food products, representing both macro-minerals (e.g., calcium, magnesium, potassium, sodium) and trace elements (e.g., zinc, iron, selenium). In the present study, T1 exhibited the highest ash value (4.53 ± 0.08%), which is suggestive of a formulation incorporating mineral-rich ingredients such as dairy proteins (whey, casein), seeds (chia, flax), or fortified powders. These ingredients not only enhance the nutritional density but also contribute to functional and stability properties of the final product. From a nutritional perspective, certain minerals play a direct role in weight management and metabolic health. For example, calcium has been shown to modulate lipid metabolism by influencing lipolysis and fatty acid oxidation, potentially enhancing weight loss in the context of a calorie-restricted diet Christensen et al. (59). Research on plant-based and dairy-based protein beverages highlights that ingredient selection and fortification strategies can markedly influence mineral density Joshi et al. (60). However, excessive mineral fortification without careful sensory optimization can sometimes produce off-flavors (e.g., metallic, chalky notes) or affect color stability, which may impact consumer appeal. Therefore, when designing low-sugar high-protein smoothies for weight management, the goal should be to achieve a balanced mineral profile — rich enough to deliver health benefits and functional stability, but without compromising sensory quality.

Carbohydrate content is a critical nutritional parameter in designing low-sugar, high-protein smoothies aimed at weight management. In the present study, T1 recorded the lowest carbohydrate content (10.33 ± 0.13%), aligning well with the low-sugar

objective and potentially offering benefits for glycemic control and satiety. Low-carbohydrate, protein-rich beverages have been shown to attenuate postprandial glycemic excursions, improve insulin sensitivity, and promote fat oxidation Gannon & Nuttall, (61). The highest carbohydrate content was observed in T3 (19.15 ± 0.20%), which may have contributed to its higher sensory acceptance. Sugars naturally mask bitterness from protein hydrolysates, enhance fruity or creamy flavor notes, and improve mouth feel by increasing body and viscosity Wiebe & Bredie (62). However, from a weight management perspective, carbohydrate inclusion should be carefully moderated to avoid undermining the calorie-reduction goal.

The smoothies maintained a near-neutral pH range (6.30–6.45), which is optimal for protein stability in dairy- and plant-protein systems, minimizing curdling and precipitation during processing and storage. The slightly higher titratable acidity (TA) observed in T1 (0.099 ± 0.002 N) likely originated from mildly acidic fruits, cultured dairy bases, or fortifying acids such as citric acid. Mild acidity is advantageous in functional beverages as it can inhibit microbial growth and extend shelf-life Sharma et al. (63). However, excessive acidity can produce a sharp or sour flavor, potentially reducing consumer acceptance, especially in sweet or dessert-type smoothies. Research in protein-fortified fruit beverages indicates that optimal pH control is achieved by blending acidic and neutral ingredients, thereby balancing microbial safety with palatability Dickinson (64).

Viscosity plays a central role in consumer perception, influencing creaminess, mouthfeel, and satiety. In this study, T3 exhibited the highest viscosity (2394 cP), most likely due to its higher fiber and protein content, which increases water-binding and forms a dense macromolecular matrix Zhu et al. (65). High-viscosity beverages have been shown to delay gastric emptying and extend satiety compared to thin liquids of equal caloric density Mattes & Rothacker (66). While viscosity can enhance functional benefits for weight management, excessive thickness may impair drinkability and reduce repeat consumption. Strategies to control viscosity include adjusting total solids, reducing fiber particle size, and optimizing homogenization pressure to achieve a balance between sensory appeal and satiety enhancement.

Storage trials revealed that T4 was the most stable formulation, showing minimal phase separation and strong aroma retention over time. In contrast, T3 exhibited

earlier separation, reflecting the challenges of stabilizing high-fat, high-protein beverages without adequate emulsification systems. Phase separation is common in protein–fat–fiber blends due to density differences, protein aggregation, and weak interfacial stabilization McClements (58). Sensory analysis identified T3 as the most liked formulation, achieving the highest scores for color, taste, flavor, texture, and overall acceptability. Its superior performance likely resulted from a balanced composition of fat, fiber, and carbohydrates, which enhanced flavor complexity, mouthfeel richness, and bitterness masking. Similar findings by Wiebe & Bredie (62) indicate that moderate sugar and fat levels improve palatability in high-protein beverages by reducing protein-related astringency and chalkiness. Conversely, T1, despite its superior nutritional profile (low sugar, high protein), received the lowest sensory scores. This suggests that nutritional optimization alone is insufficient without parallel attention to sensory design. Techniques such as natural flavor fortification, microencapsulation of sweeteners, and optimized blending of flavoring agents can bridge the gap between functional benefits and sensory enjoyment, enabling high-protein, low-sugar beverages to achieve both health goals and consumer acceptance.

CONCLUSIONS

Hence, this study has evaluated the physicochemical and sensory properties of four different groups, T1A, T2A, T3A, and T4A at 0%, 2%, 4%, and 6% almond milk concentration. However, by increasing the quantity of almond milk in the smoothie, the pH, fat, protein and physical stability of the smoothie can be altered. Thus, the consequences suggested that up to 6% of almond milk can be added in it, to enhance its properties without compromising its effectiveness. The balanced ingredients contributed to improving its shelf life and physical stability. Thus, this smoothie is a healthy product with low sugar content to manage weight

Limitation

- The physical stability of the smoothie after preparation was assessed for a short period. Due to the lack of time, no preventive measures were taken to increase its preservation time.
- The sensory panel was not based on trained members, so there might be a chance of bias.
- Smoothie preparation and analysistook place in a controlled and perfect environment.
- Only a few properties of the smoothie were assessed, while a comprehensive nutritional assessment was missing, which could be helpful in weight management.
- Only one type of smoothie with the same ingredients was assessed. There was no variety. So it could be dependent on season and the availability of all the ingredients.

Recommendations

- Future studies should focus on the increased shelf life and physical stability of the smoothie so it can be stored for some time without spoilage
- Give this weight management smoothie to the general population for use and observe the results to see if it is helpful or not.
- For the future, provide this smoothie to trained panelist members for better sensory evaluation and eliminate any bias factor present in it.
- Have a comparison in smoothies with different types of plant-based milk to better observe the health benefits in terms of weight management and nutritional availability.
- Use all-season and easily accessible fruits and vegetables to make the smoothie so they can be available anytime and everywhere.

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