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Evaluation of Replacing Titanium Dioxide with Natural Whitening Agents in Food Products, Effects on Quality and Storage Stability

Hadeel Imad Jabri

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Evaluation of Replacing Titanium Dioxide with Natural Whitening Agents in Food Products, Effects on Quality and Storage Stability

prepared by:

Hadeel Imad Jabri

B.Sc. in Nutrition and Food Processing / Hebron University/ (Hebron Palestine)

Supervisor: Dr. Ziad Ayyad

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Thesis Approval

Evaluation of Replacing Titanium Dioxide with Natural Whitening Agents in Food Products, Effects on Quality and Storage Stability

Prepared by: Hadeel Imad Jabri
Registration No: 22112808

Supervisor: Dr. Ziad Ayyad

Master thesis submitted and accepted, Date: 30-1-2024

The name and signature of the examining committee are as follows:

1. Head of The Committee: Dr. Ziad Ayyad Sign
2. External Examiner: Dr. Suleiman Alloussi Sign
3. Internal Examiner: Dr. Claude El"Ama Sign

Jerusalem-Palestine

2024\1445

Dedication

I dedicate this thesis...

This work is dedicated to martyr Muhammad Jawabreh "Abu Helmi" and to my dear parents, my brothers, sisters, and my fiancé, who have been a constant source of support and encouragement during the challenges of graduate school and life.

Declaration

I certify that this thesis submitted for the degree of master is my research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for higher degree to any other university or institution.

Hadeel Imad Jab

Signed: 

Date: 30/1/2024

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Abstract:

This research investigates different bleaching agents that can be used in food products as an alternative to titanium dioxide, which has been banned due to safety concerns. The purpose of the study is to find a substitute that can produce the same color effect as titanium dioxide, is stable during storage, is not affected by heat or acidity, and does not alter the physical, chemical, and sensory characteristics of the product.

Numerous food items have been tested for substitutes for titanium dioxide, such as Palestinian hamam bread, roasted pumpkin seeds, and chickpeas, using calcium sulfate, calcium carbonate, and rice starch. Calcium carbonate is a naturally occurring substance typically found in rocks, which is recognizable by its bright white powder form. It is frequently used in cooking as a food hardener and bleaching agent. Starch, on the other hand, is a prevalent component found in rice. When extracted from the endosperm of the rice kernel, it appears as an odorless, flavorless white powder characterized by the presence of microscopic grains. which contribute to a wide range of applications in different cooking processes and formulations. Calcium sulfate is also commonly used as a suitable bleaching agent and is permitted for use in food manufacturing, despite its low solubility in water.

The study systematically examined the impact of bleaching agents on the physical, microbial, chemical, color, sensory, moisture content, and total solids of food products during the recommended storage period. Sensory evaluations were conducted on food items to assess their flavor, color, taste, appearance, aroma, and texture. The best results were observed in products containing rice starch, followed by calcium sulfate and calcium carbonate. The performance of rice starch was better in chickpea compounds than in pumpkin seed alternatives as determined using CIE-LAB color space technology. Although no detectable bleaching agent residue was found in Fino bread after CIE-LAB analysis, rice starch was determined to be the best alternative based on sensory analysis.

This comprehensive investigation highlights the feasibility of using bleaching agents to enhance or maintain the quality attributes of various food products while adhering to safety regulations established by national food regulatory agencies.

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List of Acronyms

kg: Kilogram

gm: Gram

Min: Minute

H: hour

^{o}C: Degrees Celsius

TPC: total plate count

TC: total coliform

FC: fecal coliform

Y&M: yeast and mold

RS: reference sample

ERS: extracted rice starch

AOAC: Association of Official Analytical Chemists

BAM: Bacteriological Analytical Manual

Chapter One: Introduction

1.1. Background:

The white color is an optical illusion that occurs due to two phenomena, the first phenomenon is light scattering. Gustav Mie explained this phenomenon over a century ago and connected the scattering cross-section with the size parameter and the relative refractive index of a particle in a given matrix (deMan, PhD,1999). The second phenomenon is light reflection which was discovered by Augustin Jean Fresnel more than 200 years ago (Roland Beck,2020). Food-grade whitening agents are substances that are used to lighten or whiten food products (deMan, PhD,1999).

Regulation governs the application of whitening agents in food, with strict adherence to safety standards established by national food regulatory bodies. It is essential to verify safety information on food labels and adhere to recommended intake levels to guarantee safe consumption (Pressman, et al., 2017).

Various foods such as hummus, bread, and roasted pumpkin seeds utilize whitening agents. These agents encompass a range of substances including rice starch, calcium carbonate, calcium sulfate, and titanium dioxide (Roland Beck, 2020).

1.2. Titanium dioxide (E171)

Titanium dioxide, a naturally occurring mineral, plays a crucial function as a food additive, for its intrinsic qualities. It is also known for its brilliant white color and chemical composition; titanium dioxide proved to be an ideal candidate for imparting a vivid white hue and enhancing opacity in various food products (Ropers et al, 2017).

The safety measures of food-added titanium dioxide (TiO_2) include an analysis of recent scientific data, with details on TiO_2 nanoparticles. Although the absorption of TiO_2 particles in the digestive system is considered low, there is a possibility of their accumulation in the body (David, 2022). Dose test studies did not show any counter effects on overall status or organ toxicity. However, there have been indications of potential immunotoxicity, inflammation, and neurotoxicity (David, 2022). Concerns about genotoxicity have risen due to TiO_2 particle's capability to cause damage to the DNA. The evaluation sheds light on some uncertainties concerning threshold mechanisms, genotoxic cut-off sizes, and potential carcinogenic effects. As a result of these concerns and uncertainties, the team concluded that E 171 could no longer be considered safe as a food additive (Bischoff, *et al*., 2021).

In 2020, France and two years later, the European Union banned titanium dioxide as a food additive. However, the U.S. still allows it to be used in food products.

1.3. Calcium carbonate

Calcium carbonate ($CaCO_3$) is widely dispersed in rocks worldwide and is not limited by geological boundaries (Omari, 2016). The European Pharmacopoeia's spectral describes $CaCO_3$ as a powder that is pristine white or almost white, reflecting the purity of its molecular structure. In addition, its essence is described by the United States Pharmacopeia (USP) as a fine, tasteless, odorless, white, and microcrystalline powder (Omari, *et al*., 2016).

Based on the limited available toxicological data, calcium carbonate is not significantly toxic. Calcium is metabolized and excess is eliminated via the kidneys (Aguilar, et al., 2011)

1.3.1. Structural Formula

The chemical structure of the CaCO3) figure (1.1))

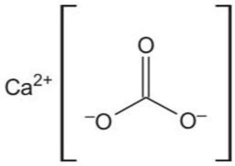


Figure 1.1 Chemical Structural of CaCO3

(Al Omari, *et al*., 2016)

1.3.1. Elemental Analysis

The theoretical values of elemental compositions of $CaCO_3$ are listed in Table (1.1)

Table 1.1

				%
Compound	Molecular formula	C	Ca	O
Calcium carbonate	$CaCO_3$	12.00	40.04	47.96

Molecular formula for CaCO3 (Al Omari, *et al.* ,2016)

1.4. Calcium sulfate

There are two types of calcium sulfate: anhydrous and dihydrate. It is added to food in order to achieve certain objectives (whitening agents, for example). It barely dissolves in water. calcium sulfate uses in food is limited by good manufacturing techniques and permitted as a food additive in most foods. (F. Aguilar, 2008) Though. Calcium sulfate ($CaSO_4$) is not highly soluble in water, it dissolves in the digestive tract well enough to function as a source of calcium. Compared to calcium carbonate, calcium sulfate has very little sensory impact. (Beck, 2020)

1.4.1. Structural Formula

The chemical structure of CaSO4 is shown in Fig. (2)

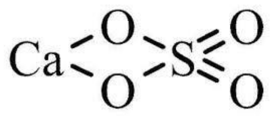


Figure 1.2. Chemical structure of $CaSO_4$

1.5. Rice starch:

Rice, considered an essential and globally impactful crop, serves as a primary source of sustenance, offering distinctive nutritional and functional qualities that meet the dietary needs of more than half of the world's population (Tester & Morrison, 1990). Out of all the complex ingredients in rice, starch is the most significant one, accounting for most of the grain's content (Wani, *et al.,* 2012). When rice starch is meticulously separated from the endosperm of the rice kernel, it appears as white color, odorless, and tasteless powder with microscopic granules. This exceptional carbohydrate is widely employed in the food industry, where its versatility and favorable attributes contribute to a multitude of culinary innovations and formulations. This underscores the pivotal role that this seemingly modest powder plays in shaping the global culinary landscape. (Bao, 2019).

1.5.1. <u>Rice starch composition</u>

The two main components of starch are amylose and amylopectin, which are made up of the same basic glucan polymers but differ in terms of branching and length. (Figure 1.3) (Amagliani, *et al*. 2016).

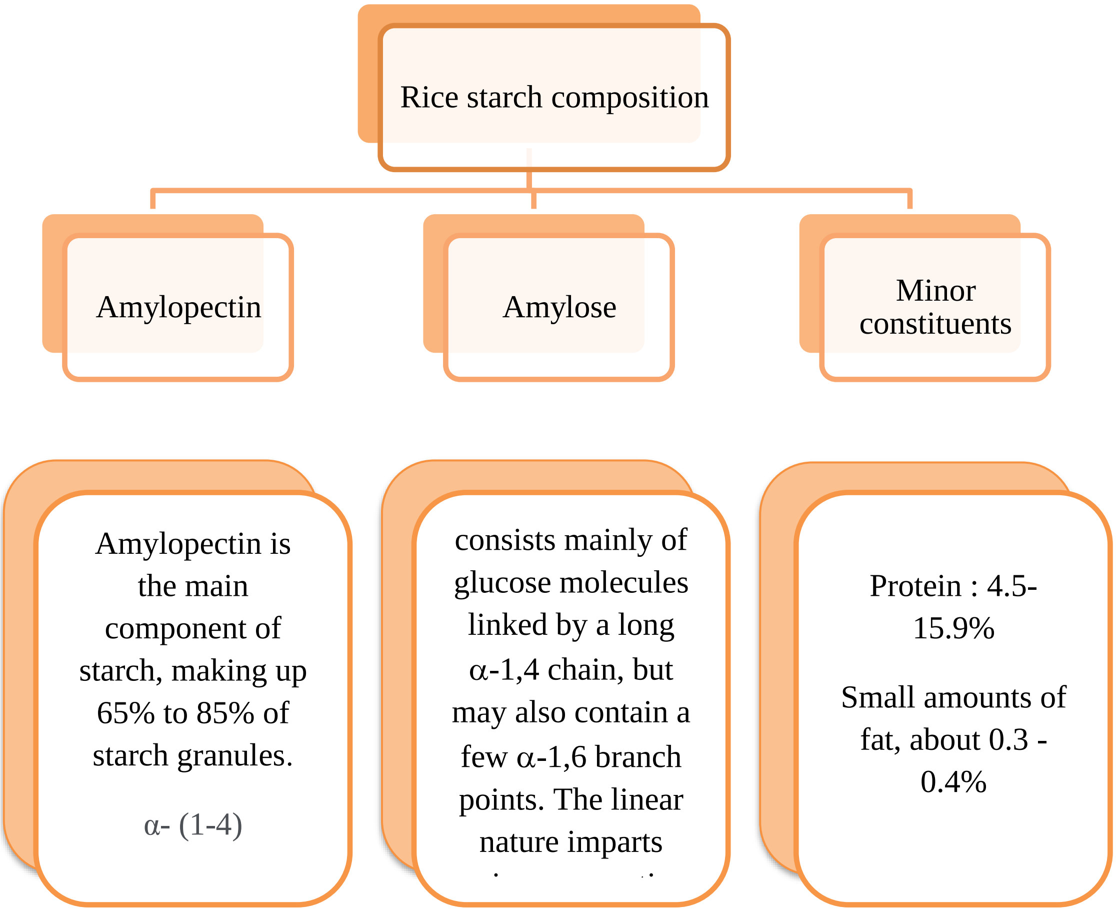


Figure 1.3. Rice starch composition (Amagliani *et al.* 2016)

1.6. **Chickpeas**

Hummus known as garbanzo beans in some regions, chickpeas assert their dominance as the most important legume within the Old-World legume family, emerging as an exceptional source of protein with implications for both gastronomic pleasure and nutritional prowess (Reister, 2020).

Humus as legumes appear to be a veritable repository of dietary fiber, polyunsaturated fatty acids, and a plethora of essential vitamins including E, C, and A, along with folic acid, iron, magnesium, and potassium. Interestingly, experimental investigations reveal a positive correlation between chickpea intake and higher scores on the Healthy Eating Index 2005 (HEI-2005), confirming the overall health benefits of incorporating these legume wonders into one's diet. (Wallace, *et al.*, 2016).

1.6.1. Types of chickpeas:

There are two main types of chickpeas: Kabuli, which has light seeds, and Desi, which has smaller and darker seeds.(Wallace, *et al.*, 2016)

1.6.2. Chickpea proteins:

Chickpeas contain two essential proteins, albumin and globulin. In addition, it contains small amounts of gluten and prolamins. Chickpea proteins have remarkable functional characteristics, including solubility, oil and water absorption capacity, emulsification, foaming, and gelling. These qualities enable them to be versatile in lots of food applications, which makes them useful components in a wide range of product formulations. (Grasso, *et al*. ,2021)

1.6.3. Chickpeas and health:

Based on recent research, Chickpeas may help with insulin, glucose, and weight control. In addition to containing dietary bioactive including phytic acid, sterols, tannins, carotenoids, and other polyphenols like isoflavones. Raw or cooked chickpeas, and hummus may potentially provide additional health advantages beyond meeting human nutrition needs.(Grasso, *et al.*, 2021).

1.7. Pumpkin seeds

Pumpkins (Cucurbit sp.), a member of the Cucurbitaceous family, is cultivated as a vegetable in many parts of the world. The protein, iron, zinc, manganese, magnesium, phosphorus, copper, potassium, polyunsaturated fatty acids, carotenoids, and c-tocopherol content of pumpkin protein concentrate and pumpkin protein isolate are starting to show (Syed, *et al.*, 2019).

The seeds have a valuable phytochemical composition that makes them beneficial for nutritional purposes. The seeds have an oil content ranging from 11% to 31%. The main fatty acids found in the seeds are stearic, palmitic, oleic, and linoleic acids (Raczyk, *et al.*, 2017).

1.8. **Palestinian hamam Bread:**

Palestinian hamam bread is recognized for its sizable loaf and bears unique regional names across the globe. In Egypt, it goes by fino, while in Saudi Arabia, it's commonly known as samli. The bread's recipe comprises fundamental ingredients such as flour, yeast, sugar, salt, and water. Nutritionally, it boasts essential nutrients, particularly carbohydrates and proteins. The incorporation of these elements enhances the bread's overall nutritional value, making it an important source of energy and protein for those including it in their diet. Moreover, the varied names across regions underscore the cultural diversity and culinary distinctions associated with this bread type. (Hussain, *et al.,* 2021)

Chapter two: Literature reviews

This chapter contains the literature reviews that were read and cited during the study.

2.1. **Rice starch:**

In 2012, Wani and colleagues conducted a study, revealing notable variations in the morphological characteristics of rice starch granules, as observed through microscopic and particle size analyses. The shapes of these granules exhibited diversity, ranging from polyhedral to irregular, and their particle sizes ranged from 2 to 7 μm. It was observed that rice starch grains are comparatively smaller than the starches found in other grains. Additionally, the amylose content in rice starch varied, ranging from virtually amylose-free in waxy rice to approximately 35% in non-waxy and long-grain rice starch.

2.2. **Color**

In 1992, Researchers Kneifel, Ulberth, and Schaffer investigated the triple reflectance method in order to assess milk and dairy products' color objectively. They made use of the CIE-LAB color parameters L*, a*, and b*. Since of this technique is crucial for figuring out the color of milk and milk products and enables exact control over food quality when producing new foods or developing existing ones.

2.3. **Chickpea:**

Yamani and Dababseh's (1994) study provide valuable insights into the microbiological quality of fresh hoummos, the investigation showed that, shedding light on seasonal variations, hygienic practices, have a potential role in inhibiting growth of microorganism contamination. The findings also contribute to the understanding of factors influencing the safety and quality of this widely consumed chickpea dip, offering implications for both consumers and the food industry.

In November 2016, Wallace and his group. indicated that, hummus is a nutrient-dense dip made from cooked and mashed chickpeas combined with tahini, olive oil, lemon juice, and seasonings. Studies show that those who include chickpeas in their diet typically consume more nutrients overall. Modest intake of hummus or chickpeas may also have other benefits, including meal nutrition improvement by slowing down the absorption of carbohydrates and prolonging stomach emptying.

In 2020, al-Qadiri *et al.* published an article entitled “The effect of irradiation with gamma rays against microbial spoilage of chickpeas preserved under refrigerated storage. In this research, they studied the effect of irradiation on the physical, chemical, and microbial properties of the product. During storage, it was noted that the pH varied after Day 10 This is because chickpeas have an acidic environment that supports the growth of various microbes.

In 2021 Grasso *et al.*, conducted a research analysis on chickpeas (Cicer arietinum L.) indicated that, chickpeas contain two essential proteins, albumin and globulin. In addition, chickpea contains small amounts of gluten and prolamins. In addition, the study highlights that, chickpea proteins exhibit commendable functional properties such as solubility, ability to absorb water and oil, emulsification, foaming, and gelling. These properties contribute to chickpea versatility in various food applications, making chickpea valuable ingredient in the formulation of various food products.

In 2023, Cui, and his co- workers, carried out a study on the mobility and thermodynamic characteristics of water in food solids, or water usability. They verified that, the presence of water in food creates an environment that is favorable for the growth of microorganisms. Thus, altering the chemical and physical characteristic of food.

2.4. Titanium dioxide:

In 2008, Younes, *et al*. evaluated the carcinogenicity of poorly soluble and poorly toxic substances: titanium dioxide and talc. Lung cancer risk was analyzed with each exposure, adjusting for several potential confounders, including smoking. Subjects with occupational exposure to titanium dioxide and industrial talc did not experience any detectable excess risk of lung cancer. The results are consistent with the recent evaluations of the IARC Monograph.

In 2020, Beck Roland investigated in his research that, there was a little support for titanium dioxide (E171) as a food additive, which have led to comparisons with other whitening agents, including calcium sulfate, magnesium carbonate, and calcium carbonate. Calcium sulfate was found the most appropriate because of its ideal particle size, natural source, and abundance of natural minerals. This research, suggests that, calcium sulfate may replace titanium dioxide as the component of choice for food whitening.

In 2021, research conducted by Ramanakumar *et al*. noted that, while TiO_2 particles exhibit low absorption through the digestive system, there is a possibility of their accumulation in the body. No toxic effects were evident at a dosage of 1000 mg E171/kg body weight/day. Nevertheless, the study observed potential immunotoxicity and inflammation linked to E 171, as well as potential neurotoxicity associated with TiO_2NPs, suggesting potential adverse effects. TiO_2 particles were found to induce DNA strand breaks and chromosomal damage, although genetic mutations were not observed.

In 2021, Roland Beck published a paper in which he began looking for a bleaching chemical that the food sector might use instead of titanium dioxide. A multitude of alternatives are available, each with unique attributes that restrict their suitability. The best option in terms of applicability, regulatory approval, and cost-effectiveness is the insoluble form of anhydrous calcium sulfate, which addresses stability issues related to carbonates and starch.

2.5. Sensory evaluation:

In 2010, Lawless and Hyman analyzed the food industry's use of sensory analysis to evaluate food products. Their research offered a thorough examination of the scientific techniques utilized to quantify and comprehend the sensory qualities of food, such as texture, color, scent, taste, and look. The study focused on the various uses of sensory evaluation, ranging from consumer-focused product development to quality control. The study highlighted sensory analysis critical role in guaranteeing the prosperity and contentment of producers and customers in the dynamic food business.

2.6. Bread :

In 2014, Fadda *et al.* studied the condition known as "bread stalling" which occurs when bread gradually gets firmer and drier, decreasing its shelf life. Since the rearrangement of starch molecules is the primary cause of bread hardening, this process is influenced by various factors, including starch degradation. Since the amount of amylose to amylopectin in the flour affects how quickly bread hardens, moisture loss also plays a role in the process. High levels of amylose hasten the stalling process. Bread can be kept fresher for longer by keeping it in airtight containers, which also helps to slow down the staling process.

In 2019, Amal M. H. and Abdel-Haleem observed the beneficial effect of dry heat treatment on wheat bran and wheat germ, mitigating the undamaging effects on dough rheology while enhancing the nutritional and sensory properties of Fino bread. Sensory evaluations indicated significant improvements in taste, flavor, and overall acceptability scores for loaves containing hot particles, indicating that consumers prefer the improved qualities resulting from dry heat processing.

Hassan *et al.* (2020) examined the production of gluten-free Fino bread that was palatable and suitable for celiac sufferers by applying locally available components that have nutritional advantages. The urgent demand for a suitable gluten-free Fino bread for kids with celiac illness was effectively met by their studies of sensory evaluations, nutritional analyses, and rheological evaluations.

2.7. Pumpkin seed:

In their 2017 study conducted by Raczyk *et al.*, investigated the impact of roasting and storage on pumpkin seeds and their influence on the characteristics of cold-pressed pumpkin seed oil, renowned for its unique flavor and perceived health advantages. Utilizing analytical techniques, the findings indicated that the fatty acid composition remains unaffected by the roasting and storage processes. However, alterations were observed in phytosterols and tocopherols, coupled with a reduction in carotenoid content following storage. The roasted oil exhibits a darker hue that undergoes notable modifications over the storage period. Generally, the physicochemical properties and oxidative stability of oil derived from roasted seeds surpass those of oil from unroasted seeds.

In 2021, Peng *et al.*, examined the crucial significance of plant-based diets in tackling environmental and health issues. The authors emphasized the nutritional benefits of pumpkin seeds, which are abundant in essential micronutrients, oil, and protein. The investigation delved into the effects of roasting pumpkin seeds at different temperatures on protein levels, fatty acid content, and antioxidant properties. The results indicated that roasting at 160°C produced the highest-quality protein. As revealed by the study, these findings could potentially improve the nutritional value of pumpkin seeds and encourage their incorporation into vegetarian diets.

Statement of the problem:

Titanium dioxide is traditionally used as a bleaching agent in food products, but titanium dioxide (also known as E171) has recently been banned from use in foods and nutritional supplements in Europe and many other countries, so an alternative use must be provided.

Hypothesis:

1. Rice starch, calcium sulfate, and calcium carbonate are alternatives to titanium dioxide in various food products.
2. Calcium sulfate, calcium carbonate, and rice starch do not affect the sensory and chemical properties.
3. Calcium sulfate, calcium carbonate, and rice starch are not affected by differences in temperature and pH of food products.
4. Calcium sulfate, calcium carbonate, and rice starch do not affect the shelf life of food products.

Objectives of the study:

1. Searching for an alternative to titanium dioxide that is globally recognized as safe and acceptable for health.
2. Finding an alternative that gives the same effect as titanium dioxide on color and darkness.
3. Determine the extent to which the substitute affects the stability of the product during storage.
4. Determine the effect of the alternative on pH in different environments.
5. Determine the extent to which the alternative is affected by heat.
6. Determine the extent to which the alternative affects the physical and chemical properties of the product.
7. Ensure that the substitute is inactive to other ingredients such as flavours, smell, appearance or taste.
8. Ensure that the bleaching agents do not experience any unwanted sensory effects.

Chapter Three: Methodology

3.1. Samples

3.1.1. Chickpeas.

3.1.2. Bread.

3.1.3. Pumpkin seeds.

3.2. Rice Starch extraction

3.2.1. Preparation of rice starch sample

Materials and Tools:

The necessary materials and tools were gathered, including distilled and sterilized water, rice, an oven with air circulation, two bowls, a strainer (0.149/100 mm mesh), a mixer, and a scale.

Method:

1. The rice was washed and weighed.
2. Rice was soaked in water (1:2 rice: water) in an industrial blender for 5 minutes.
3. The fractionated material was filtered to separate the residue, which was further broken down and filtered again.
4. The filtered liquid was clarified using a strainer (100/0.149 mm mesh) and left on a bench for 24 hours to allow the starch to decant.
5. The starch was then dried in an oven with air circulation at 45°C for 10 hours and stored in plastic bags, in darkness. (Figure (1.3)). (Ramos da Silva, Luan, 2020)

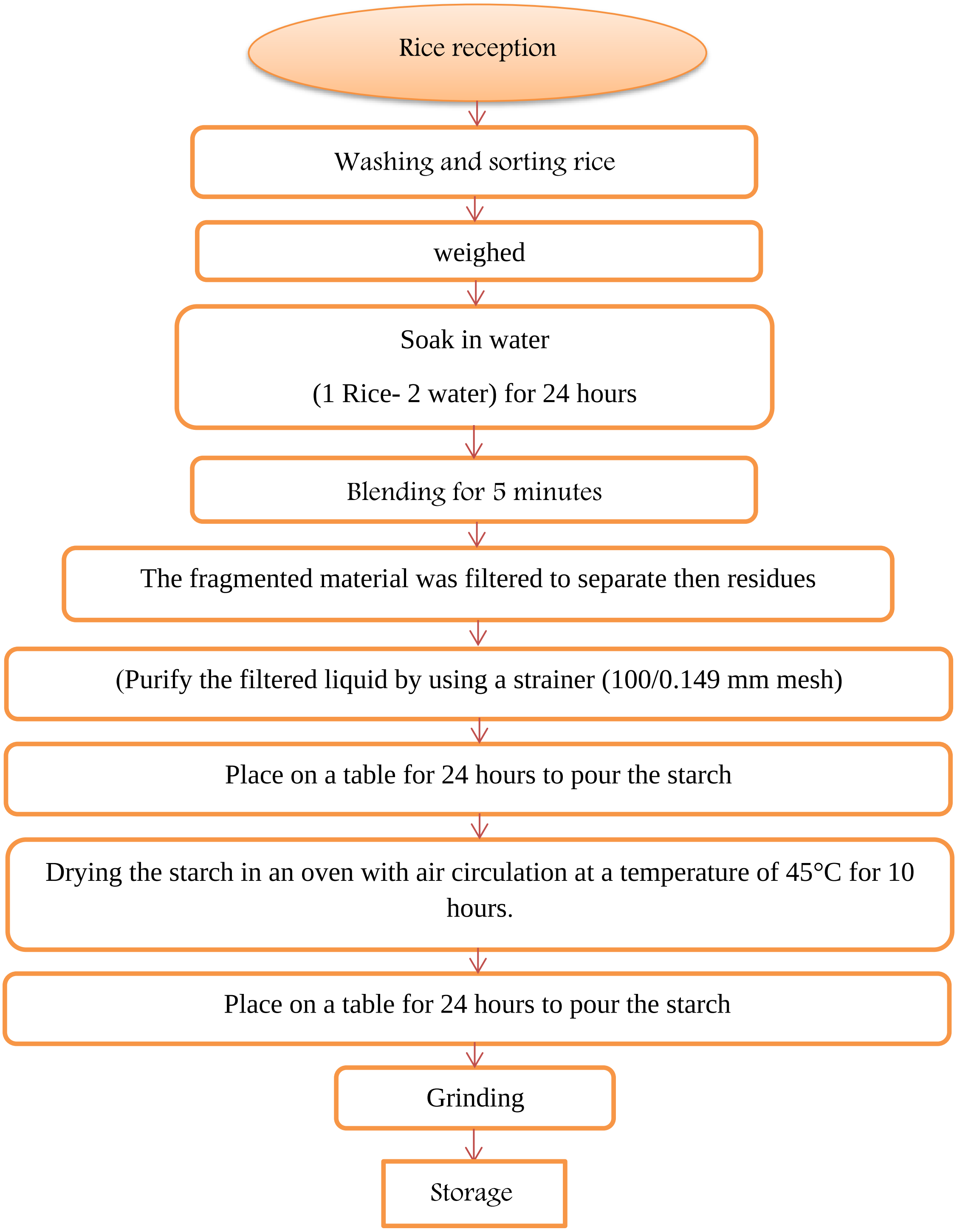


Figure 3.1 Rice starch extraction

(Da Silva, 2020)

3.3. Chickpeas samples preparation:

Materials and tools:

The necessary materials were prepared, such as chickpeas with spices, starch, salt, tahini, oil, ice, lemon, and whitening agents. The necessary equipment was also prepared, such as a bowl for mixing materials

Method:

- ✓ The chickpeas were received, cleaned, sorted, and immersed in water for about fourteen hours.
- ✓ The chickpeas were then cooked in a pressure cooker (C22-6L model) for 30 minutes.
- ✓ After cooling to 4 to 9 degrees Celsius, chickpeas were mashed with oil, tahini, spices, salt, and different whitening agents in a (Multi Grinder/MG-2021).
- ✓ The finished product was then filled into PET plastic containers and held for additional evaluation in a fridge, as can be seen in Figure (2.3).

Table 3.1 Mix of Hummus Dip

	Added whitening agents	**Quantity added**
Control	Control Sample (RS) plan hummus	Without adding whitening agents
R.S	Contains Extracted rice starch (ERS)	2%
TiO_2	Contains titanium dioxide	0.2%
$CaSO_4$	Contains calcium sulphate	3%
$CaCO_3$	Contains calcium carbonate	4%
All quantities are referenced to the Codex Alimentarius and Palestinian standard		

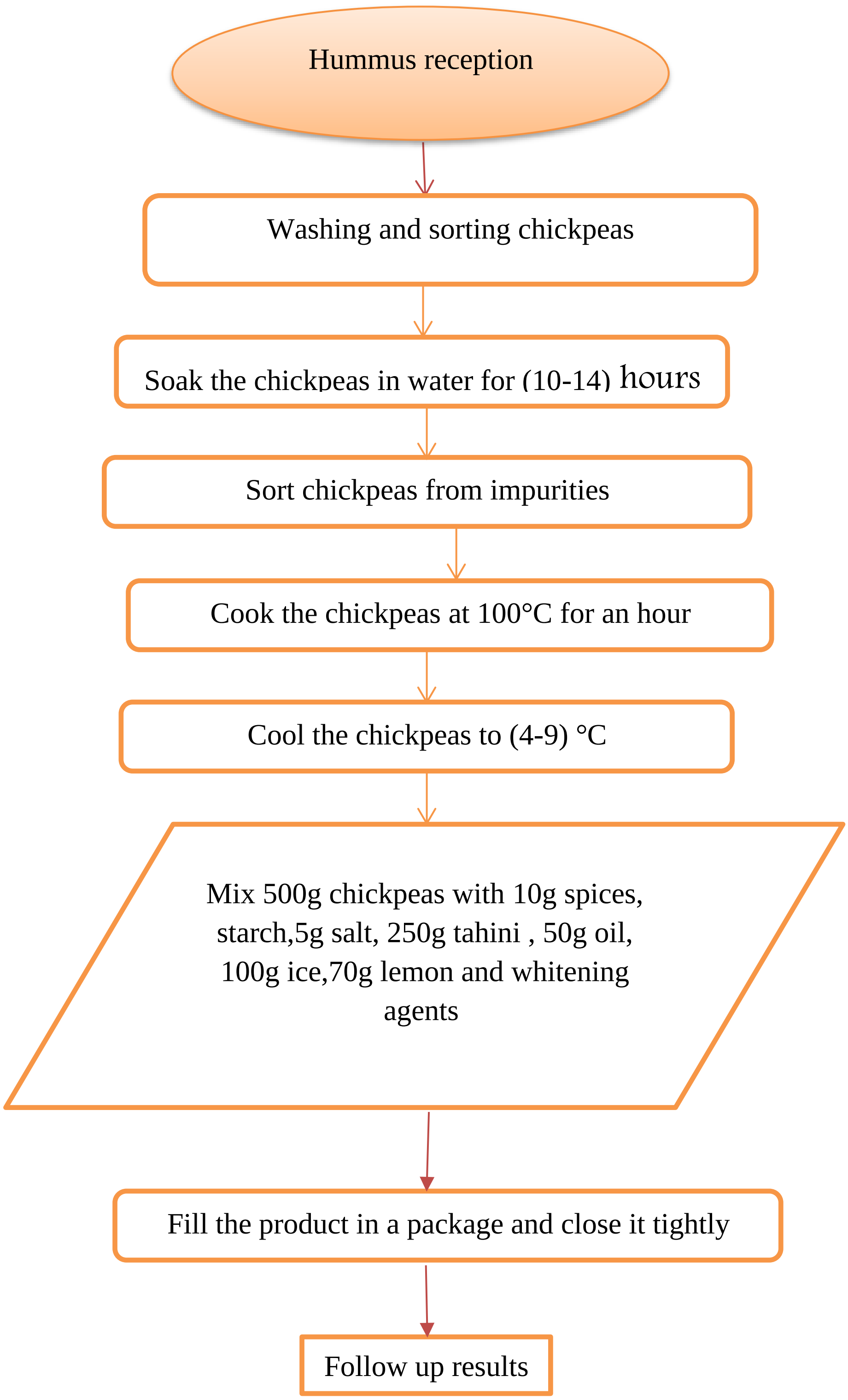


Figure 3.2 Hummus (chickpeas) manufacturing process

3.4. Preparation roasted pumpkin seeds

<u>Materials and tools:</u>

The necessary materials were prepared, such as pumpkin seeds, cornstarch, whitening agents, salt, and sterile distilled water. The necessary equipment was also prepared, such as a bowl for mixing materials and a roasting oven for seeds.

<u>Method:</u>

1. The seeds were received, their cleanliness was verified, and all tests were examined. The tests were as follows:
   - ✓ Sensory tests: taste, color, texture, smell.
   - ✓ Microbial tests: total coliform, fecal coliform, yeast and mold, Total plate count.
   - ✓ The components: carbohydrates, protein, fat, ash, sodium.
   - ✓ Moisture content.
   - ✓ Total solid.
2. The seeds were sorted and then a solution was prepared consisting of water, salt, corn starch, and whitening agents.
3. Roasting the seeds at a high temperature.
4. The final product was examined with all necessary physical, chemical and microbial tests.
5. Follow the product.

Table (3.2), Samples of rosted pumpkin seeds.

	Added whitening agents	**Quantity added**
Control	RS	Without adding whitening agents
R.S	Addition rice starch	4%
TiO_2	Addition titanium dioxide	2%
$CaSO_4$	Addition calcium sulphate	5%
$CaCO_3$	Addition calcium carbonate	6%
All quantities are referenced to the Codex Alimentarius and Palestinian standard		

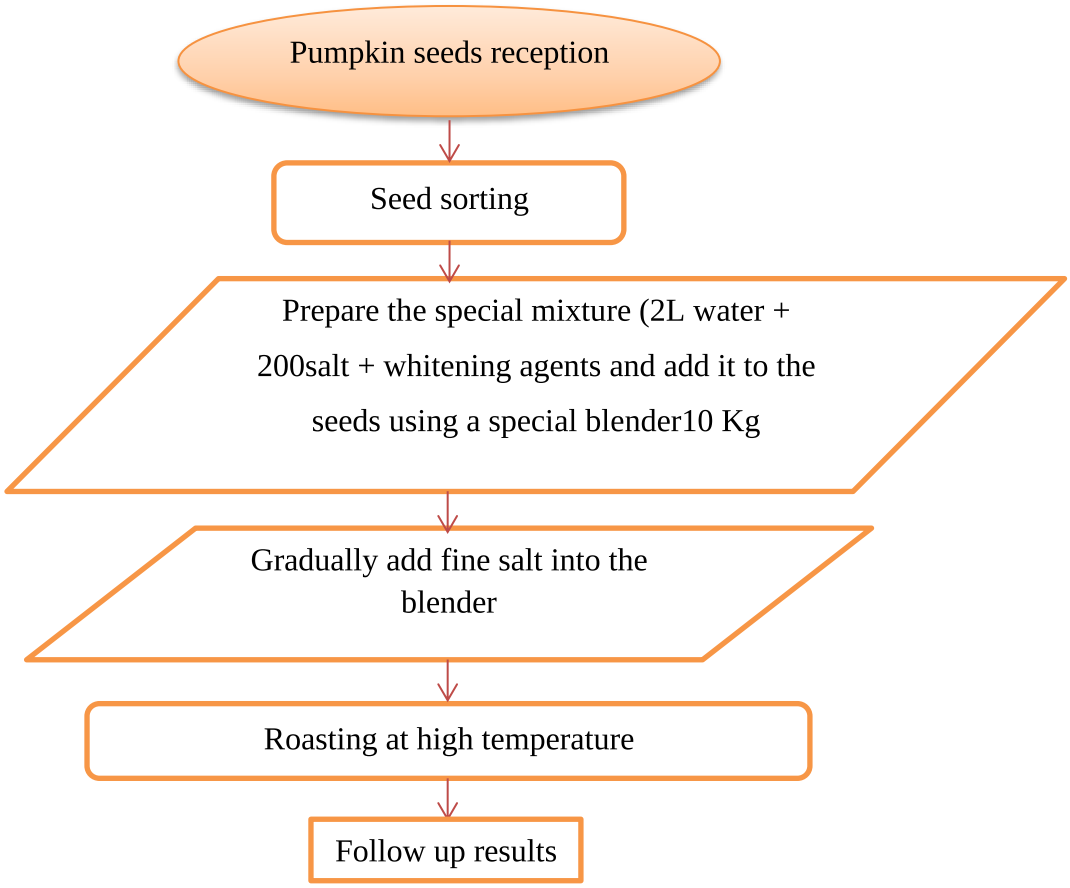


Figure 3.3 Flow chart of roasted pumpkin seeds

(Peng, *et al.*, 2021)

Preparation of a hamam bread sample

Materials and tools:

The necessary materials were prepared, such as flour, salt, whitening agents, oil, yeast, sugar, and sterile distilled water. The necessary equipment was also prepared, such as a kneading bowl, a baking tray, and an oven.

Method:

1. The Raw materials were received, their cleanliness was verified, and all tests were examined. The tests were as follows:
   - ✓ Sensory tests: taste, color, texture, smell.
   - ✓ Microbial tests: total coliform, fecal coliform, yeast and mold, Total plate count.
   - ✓ The components: carbohydrates, protein, fat, ash, sodium.
   - ✓ Moisture content and total solid.
2. In a clean bowl, put the flour, warm water, sugar, yeast, vegetable oil, vinegar and salt.
3. Kneaded for 10 minutes.
4. The dough was left to ferment for an hour.
5. The dough was cut into thin pieces, then rolled lengthwise and left for 20 minutes.
6. It was baked at 200°C for 20 minutes

Table 3.3: samples of Palestinian hamam bread

	Added whitening agents	**Quantity added**
Control	RS	Without adding whitening agents
E.R.S	Contains rice starch	4%
TiO_2	Contains titanium dioxide	2%
$CaSO_4$	Contains calcium sulphate	5%
$CaCO_3$	Contains calcium carbonate	6%
All quantities are referenced to the Codex Alimentarius and Palestinian standard		

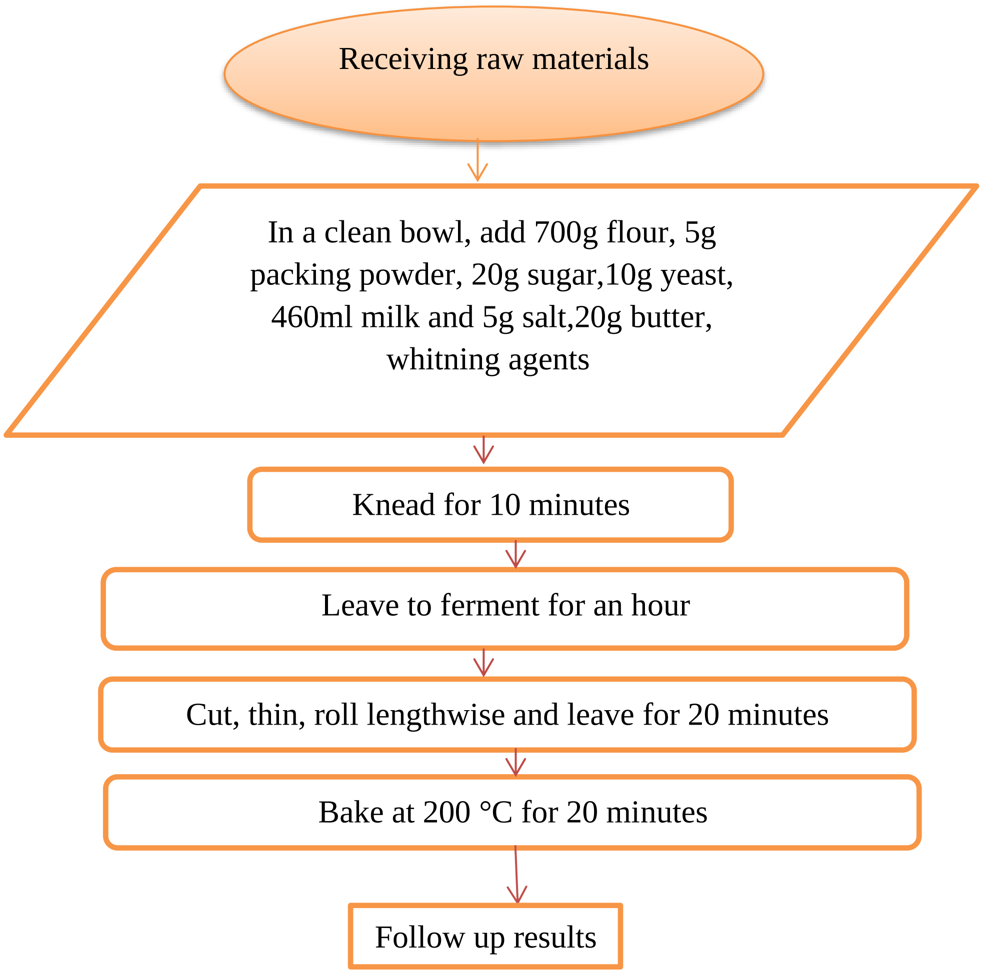


Figure 3.4 Steps Palestinian hamam bread was baked using Certified Method 10-10 by AACC International (2003).

(Enayat M. Hassan, 2020)

3.5. **Products follow-up**

Bread was examined for 7 days, each sample from pumpkin seed and hummus products were examined for a one month (30 days) for their microbial, chemical and physical quality characteristics as follow.

3.5.1. Physical and microbial evaluation:

Samples of the two products, hummus, and pumpkin seeds were analysed every 10 days. Regarding Palestinian hamam bread, testings were carried out twice: once right after it was prepared and a second time seven days later, as shown in Figure (3.5).
All microbial analyses were conducted according to the FDA- BAM manual of analysis (ref. ref)

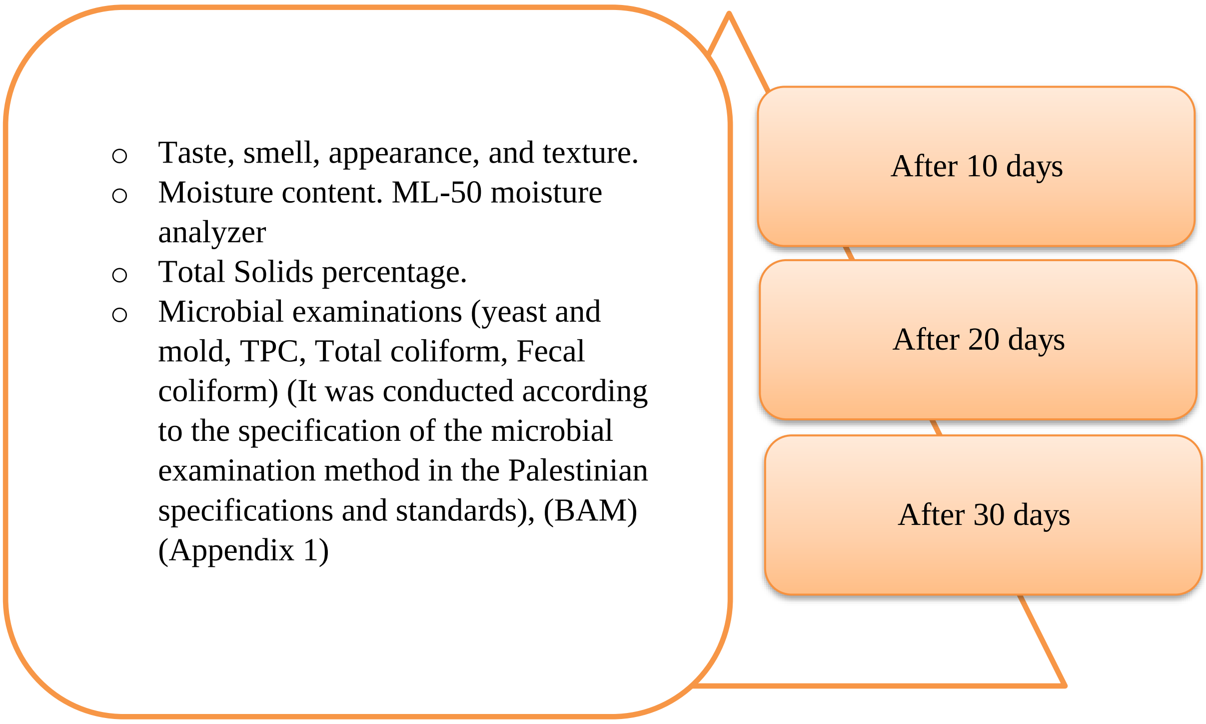


Figure 3.5 Tests performed for products during follow-up

3.5.2. Chemical analysis:

The protein content was examined by the Kjeldahl method (AOAC 1997), the fat content was determined by the Soxhlet method (AOAC 963.15), and the ash content was determined by the dry method (AOAC 936.07) using a muffle furnace (HERATHERM oven model). Chickpea samples were analyzed for the pH (AOAC method 981.12), for the carbohydrate value, it was calculated by using the equation (100- (fat% Protein%, ash% and water%)) (Appendix 1)

3.5.3. Data analysis:

The information was presented as mean ± SD, and the statistical examination involved employing one-way analysis of variance (ANOVA) followed by LSD's Fisher test. A p-value below 0.05 ($p < 0.05$) was considered indicative of statistical significance. The analysis was conducted utilizing Excel Stat software, specifically (version 2.03). Letters (a-c) in each table and figures in the result section indicates the statistical differences between respective samples

3.5.4. Sensory evaluation:

The most important factor in determining whether a consumer will accept a product is its sensory behavior such as the food products' flavor, texture, and taste satisfy the requirements. (Morten Meilgaard, 1999)

Eight experts were chosen to conduct the sensory assessment. The product-appropriate containers were used to hold the samples; for instance, BET plastic bags were used for bread and pumpkin seeds, and BET plastic containers were used for hummus. (Appendix 1)

All food samples, including Reference sample (RS) and whitening agent-containing samples, were assessed. The following characteristics of hummus were investigated: texture (creamy or hard), coloration (light or dark), smell (natural or abnormal), and flavor (sour, mild, salty, or bitter). Pumpkin seed's texture (moist or crunchy), color (light or dark), odor (natural or artificial), and appearance, were taken into consideration. Lastly, the characteristics of Palestinian hamam bread: texture (hard or spongy), color (light or dark), appearance, and taste (moderate, salty). (Enayat M. Hassan, 2020).

3.5.5. Color analysis:

The CIE-LAB system was used. The system uses the values of L*, a*, and b* to determine the position of the sample on the color axis. (Appendix 1) The L* value indicates how light or dark it is, while the a* value indicates green with negative values and red with positive values. On the other hand, b* indicates blue with negative values and yellow with positive values (Spyridon E. Papadakis, 2000), (W Kneifel1992 ،).

4.2. Chickpeas dip (hummus)

4.2.1. Effect of adding whitening agents on the physical properties of hummus

4.2.1.1. Moisture content and total solid:

Throughout the storage period, there was a significant increase in the percentage of total solids, while the moisture content of the hummus decreased significantly. This trend was consistent in both the control sample and all samples containing bleaching agents, as detailed in Table 4.2. These changes can be attributed to the adsorption process, which is characteristic of this type of food that tends to lose moisture during storage. (Cánovas, *et al.*,2007).

	Moisture Content (%)	Total Solids (%)	pH
Control	30.133±0.5 a	69.867±0.5 c	4.400±0.05 a
Control after 10 days	29.900±0.3 b	70.100±3 b	4.233 b
Control after 20 days	29.833±0.3 b	70.167±0.3 b	3.833±0.05 c
Control after 30 days	29.467±0.07 c	70.533±0.05 a	3.800±0.05 c
E.R.S	32.000±0.58 a	66.5±0.5 b	4.5 a
E.R.S after 10 days	31.933±0.1 a	67.1±1 a,b	4.3 ±0.05 b
E.R.S after 20 days	31.800±0.1 a	67.5±0.1 a	4.1 ±0.05 c
E.R.S after 30 days	30.300±0.05 b	67.8±0.05a	4.03±0.05 d
TiO_2	32.900 ±0.5 a	67.100 ± 0.5 b	4.500±0.05 a
TiO_2 after 10 days	32.767 ±0.2 a	67.233 ± 2 b	4.333±0.05 b
TiO_2 after 20 days	32.500 ±0.2 a.b	67.500± 0.2 a.b	4.133±0.05 c
TiO_2 after 30 days	32.133±0.06 b	67.867± 0.05 a	4.033±0.05 d
$CaSO_4$	33.050±0.1 a	66.950± 0.1 c	4.467±0.05 a
$CaSO_4$ after 10 days	32.350±0.05b	67.650±0.05 b	4.333±0.05 b
$CaSO_4$ after 20 days	31.983±0.1 b	68.017± 0.1 b	4.300±0.05 b.c
$CaSO_4$ after 30 days	31.417±0.26c	68.583± 0.26 a	4.233±0.05 c
$CaCO_3$	33.200±0.15 a	66.800± 0.1527 c	4.533±0.062 a
$CaCO_3$ 10 day	32.200±0.1 b	67.800±0.1 b	4.500±0.057 a
$CaCO_3$ 20 day	31.833±0.05 b	68.167±0.0577 b	4.367±0.0577 c
$CaCO_3$ 30 day	30.067±0.1 c	69.933±0.1527 a	4.267±0.0577 c

Table 4.1 Results of chemical and physical tests for hummus from day 0 to day 30

4.2.1.1. pH:

The pH showed a significant decrease in all hummus samples, including the control sample, during the storage period from day 0 to day 30, as shown in Table (4.1). Since the change in pH occurred in the control sample as well, this can be explained by the fact that microbial growth leads to a decrease in pH (Hamza Al-Qadri, 2020) (Al-Yamani and Al-Dabbasa 1994)

4.2.3 Microbial analysis

All chickpea samples exhibited a significant increase in the total plate count over the 30-day storage duration. Notably, there was a marked rise in the total platelet count (TPC) from day 0 to day 30 of storage across all chickpea samples tested. This phenomenon can be attributed to the favorable conditions for microbial growth present in chickpeas, characterized by their moisture content and rich nutrient composition. Furthermore, no detectable growth of total coliforms, fecal coliforms, yeast, or molds occurred during storage, as indicated in Table 4.2. The presence of TPC suggests potential issues with manufacturing processes or cross-contamination, with microbial proliferation potentially occurring during production, contingent upon the microbial environment (Hamza Al-Qadri, 2020).

Table 4.2 Microbial number for hummus samples

	TPC (cfu)	TC (cfu)	FC (cfu)	Y&M(cfu)
Control	250±1 a	0	0	0
Control after 10 days	260±3 a	0	0	0
Control after 20 days	290±3 b	0	0	0
Control after 30 days	320±3 c	0	0	0
R.S	230±1 d	0	0	0
R.S after 10 days	260±1 c	0	0	0
R.S after 20 days	290±1 b	0	0	0
R.S after 30 days	320±1 a	0	0	0
TiO_2	240±1 d	0	0	0
TiO_2 after 10 days	260±2 c	0	0	0
TiO_2 after 20 days	290±2 b	0	0	0
TiO_2after 30 days	320±2 a	0	0	0
$CaSO_4$	150±1 a	0	0	0
$CaSO_4$ after 10 days	180±1 b	0	0	0
$CaSO_4$ after 20 days	200±1 c	0	0	0
$CaSO_4$ after 30 days	250±1 d	0	0	0
$CaCO_3$	200±1 d	0	0	0
$CaCO_3$ 10 day	230±1 c	0	0	0
$CaCO_3$ 20 day	250 b	0	0	0
$CaCO_3$ 30 day	280±1 a	0	0	0

4.2.2. Effect of whitening agents on the physical and chemical properties of hummus at 0 days

4.2.2.1. Moisture content and total solid

The control sample demonstrated the least amount of total solids, whereas a considerable significant increase in total solids was noted in the two formulations (R.S and TiO_2). Conversely, calcium carbonate ($CaCO_3$) exhibited a discernible increase compared to the other samples. These findings can be ascribed to the differing proportions of additives incorporated into each sample during the preparation phase. The proportions were carefully determined, with the reference sample remaining devoid of additives by the stringent guidelines outlined by the Codex Alimentarius.

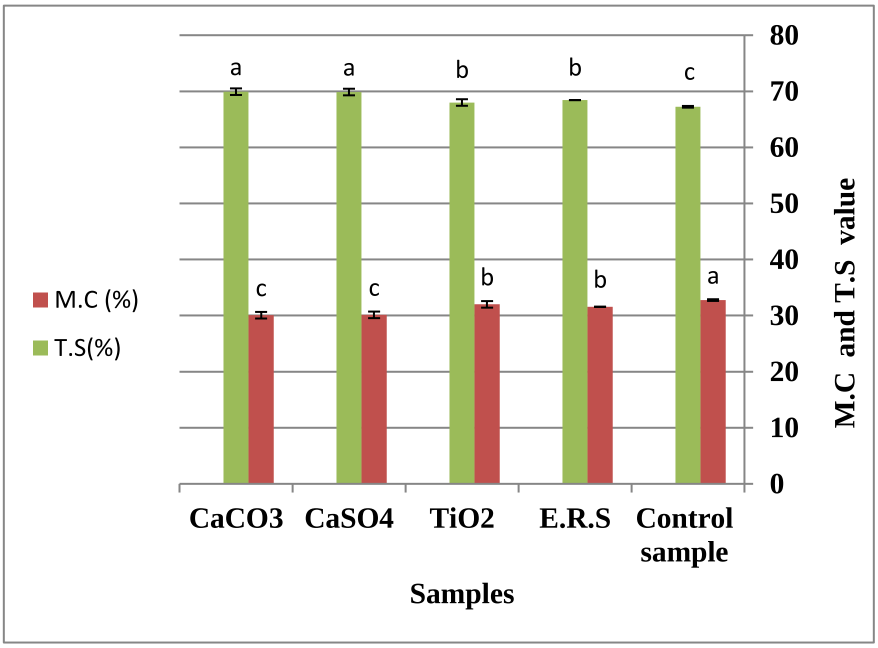


Figure 4.1 moisture content and total solids for hummus with different whitening agent in 0 day

4.2.2.2. pH:

During the pH test, a product containing whitening agents such as rice starch, titanium dioxide (TiO_2), calcium sulfate ($CaSO_4$), and calcium carbonate ($CaCO_3$) was analyzed. The results showed that adding these agents did not significantly affect the pH of the product. The pH levels were measured at 4.5, 4.46, 4.46, and 4.53, respectively. The sample with the lowest pH was the RS at 4.40, while the highest pH was observed in the samples containing calcium carbonate. Figure (4.1) This can be explained by the fact that calcium carbonate is a basic compound that slightly increases the pH level. (Hamzah Al-Qadiri, 2020**)**

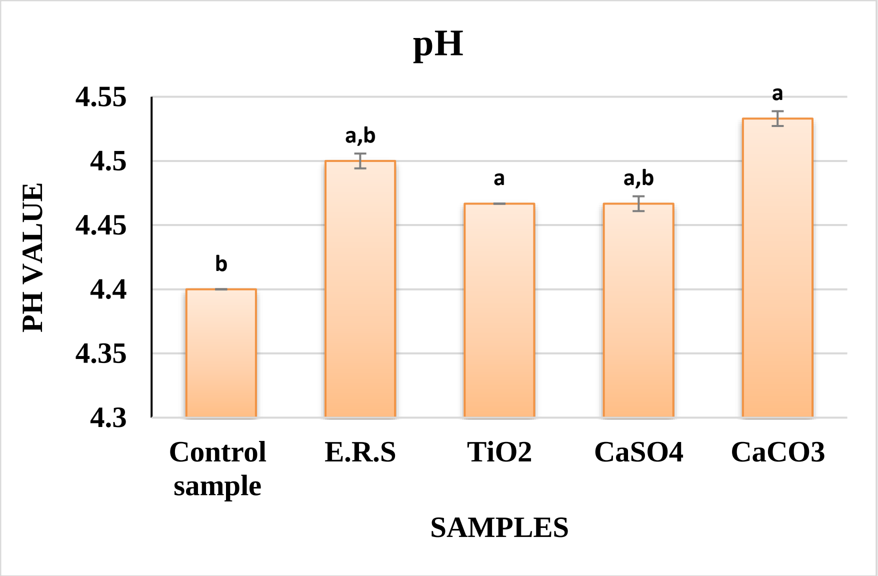


Figure 4.2 PH for hummus with different whitening agent in 0 day

4.2.3. Results of microbial tests for hummus for days 0

Examination revealed the absence of mold, fecal coliforms, or total coliform growth. Nevertheless, as illustrated in **Figure 4.3**, there is a rise in the overall plate count (TPC). The number of microorganisms in every sample, encompassing the control sample and those with bleaching agents, exhibited significant variation. Considering the conducive environment for microbial proliferation in chickpeas, such as elevated moisture and nutrient levels, this is presumably due to inadequate processing or cross-contamination (Yamani & Mehyar, 2011).

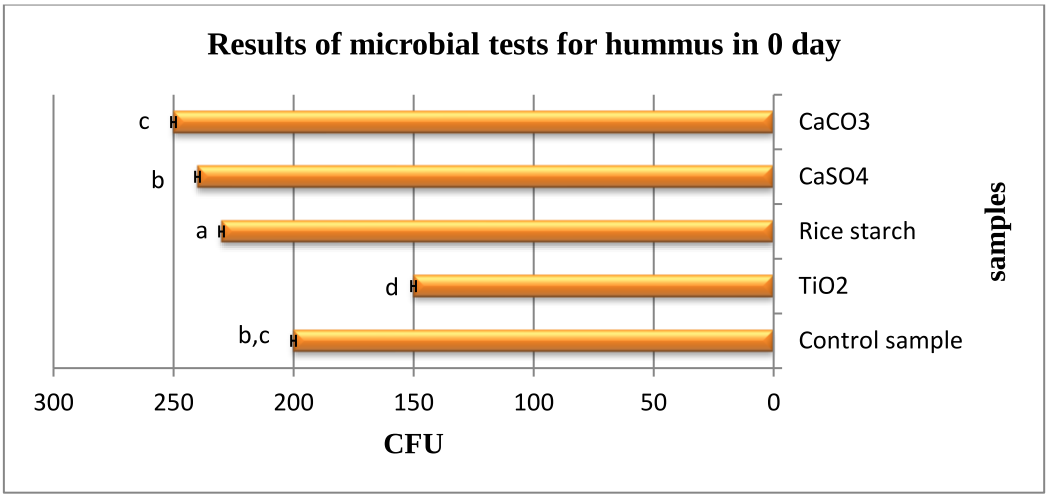


Figure 4.3 Microbial growth for hummus with different whitening agent at 0 day

4.2.4. Physical and Chemical properties of hummus after 30 days

4.2.4.1. Moisture content and total solid:

The control sample and the sample with TiO_2 exhibited the highest moisture content among all samples. Nevertheless, there was no discernible contrast between them, nor was there any significant distinction between the two samples (E.R.S and TiO_2), followed by the sample containing calcium sulfate, with $CaCO_3$ displaying the least moisture content among the five formulations (as illustrated in Figure 4.4). This variance can be elucidated by the differences in the quantities of bleaching agents added, as they were incorporated by the respective requirements and in compliance with Codex Alimentarius standards and guidelines.

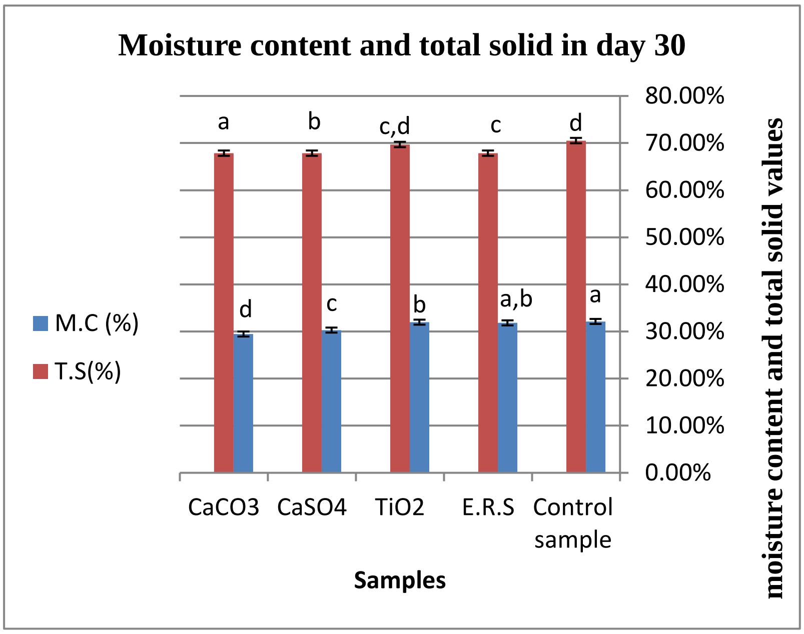


Figure 4.4 Effect of whitening agents on the moisture content and total solid of hummus in day 30

4.2.4.2. pH**:**

While all formulations incorporating bleaching agents displayed elevated pH levels compared to the control sample, products containing calcium carbonate exhibited consistently high pH values, akin to those containing calcium sulfate. This alkaline tendency can be attributed to the additive's composition (Al-Omari, 2016). Furthermore, variations in pH among the products may be elucidated by discrepancies in microbial counts and their impact on the formulations (Yamani & Mehyar, 2011).

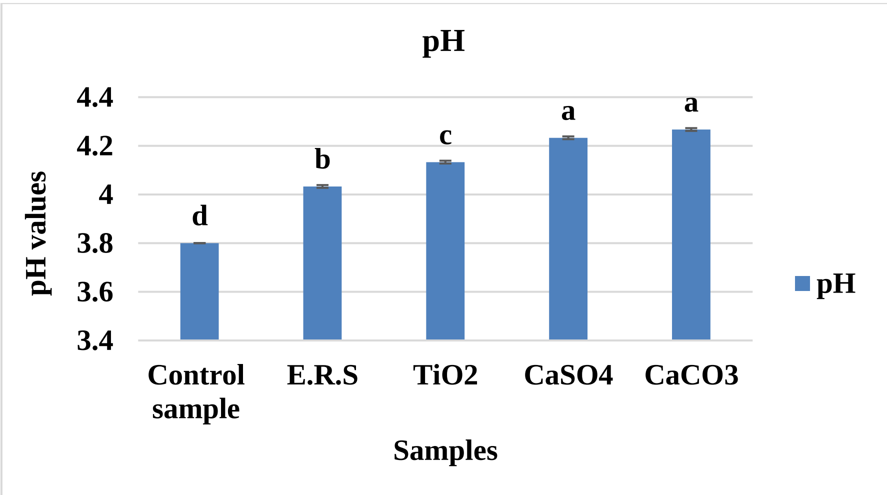


Figure 4.5 Effect of whitening agents on the pH of hummus on day 30

4.2.5. Results of microbial tests for hummus for day 30

No growth was found in total coliforms, fecal coliforms, yeast & molds. However, growth was detected in the total plate count (TPC), as shown in Figure 4.6. Microbial counts showed significant differences between samples, including the RS sample and samples containing bleaching agents. This is likely due to poor processing or cross-contamination, as chickpeas provide a favorable environment for microbial growth (Al-Qadiri, 2020).

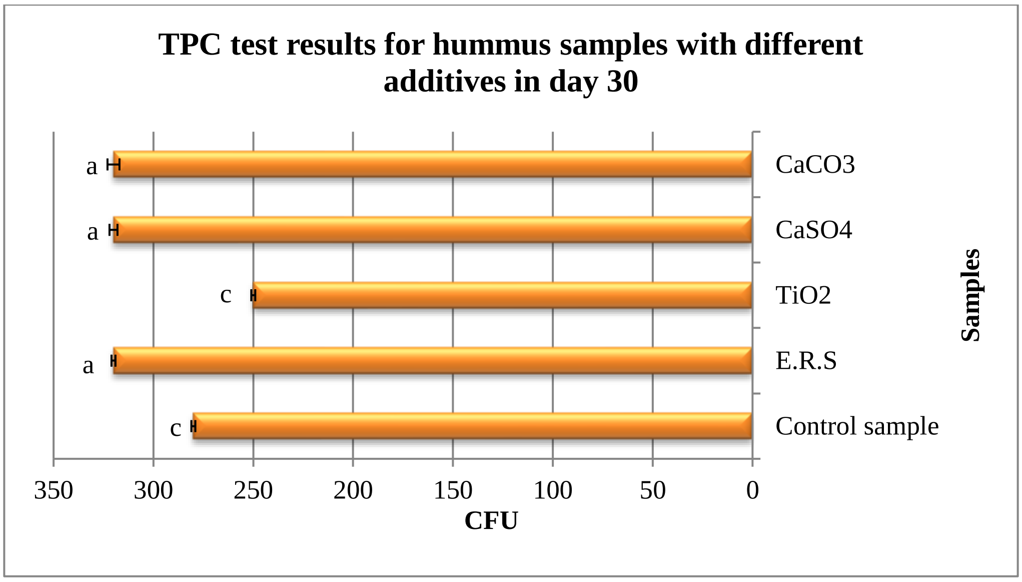


Figure 4.6 Results of microbial tests for hummus in day 30

4.2.6. **The effect of adding whitening agents on the color of the hummus product**

The CIE-LAB system uses the L*, a*, and b* values to determine the position of the sample on the color axis. The L* value indicates how light or dark the sample is, while a* value indicates green with negative values and red with positive values. On the other hand, b* indicates blue with negative values and yellow with positive values (Kneife, 1992). (Papadakis, 2000).There were no significant differences in L values, Figure (4.7) which is expected given that the result is off-white rather than dazzling white. On the other hand, it was found that (control) is the darkest among the samples, followed by $CaCO_3$ and $CaSO_4$, respectively, and finally, the two formulas (R.S and TiO_2), which are the lightest samples in color. Figure (4.7).

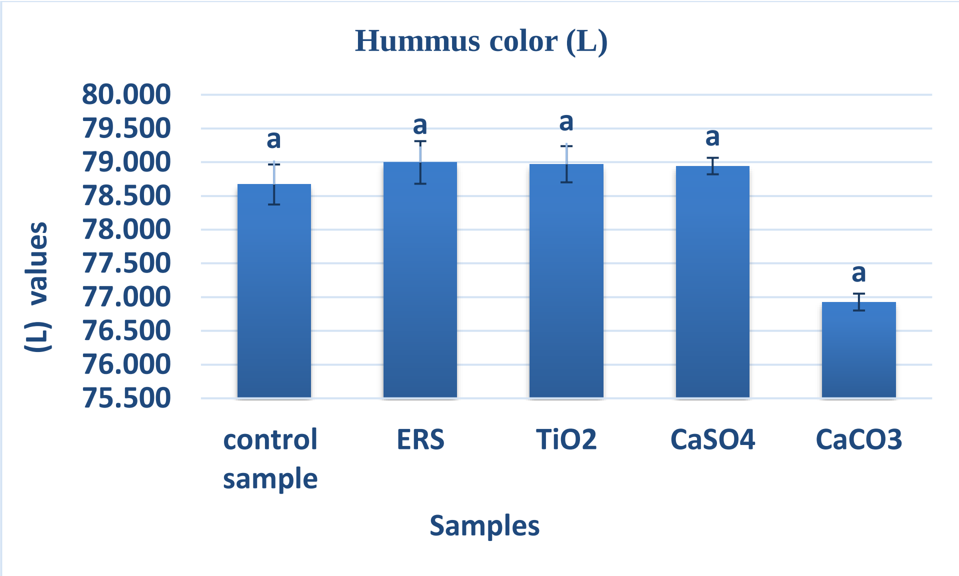


Figure 4.7 Color value (L) white color of hummus with 5 whitening agents

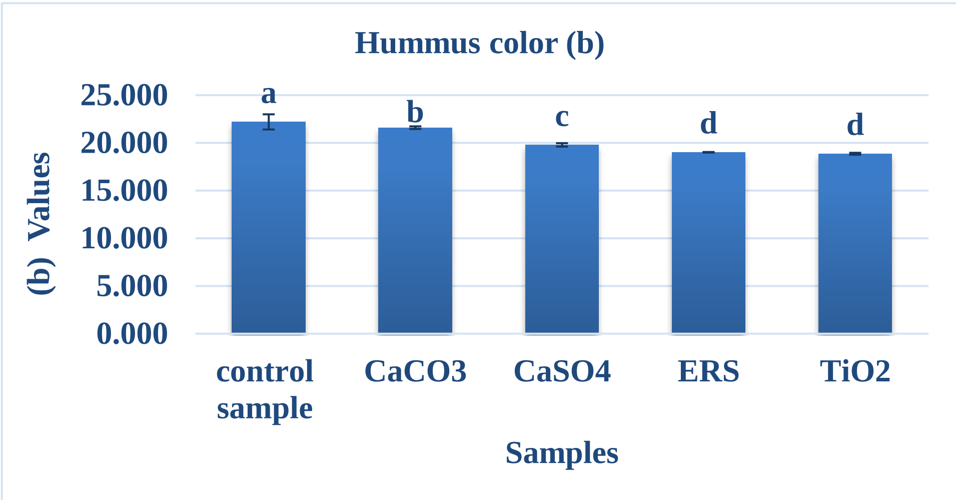


Figure 4.8 Color value (b) yellow color of hummus with 5 whitening agents

Comparing the results of the red color values (a), it was noted that the red color values were low, ranging between 0.063 and 2.630, Figure (4.9). This result indicates that there is little red color in the chickpea samples. As there is a prominent difference between the formulas, the reddest in the samples was $CaSO_4$, followed by $CaCO_3$. As for the two formulas (R.S and TiO_2) and finally Control , it is the least red among the samples.

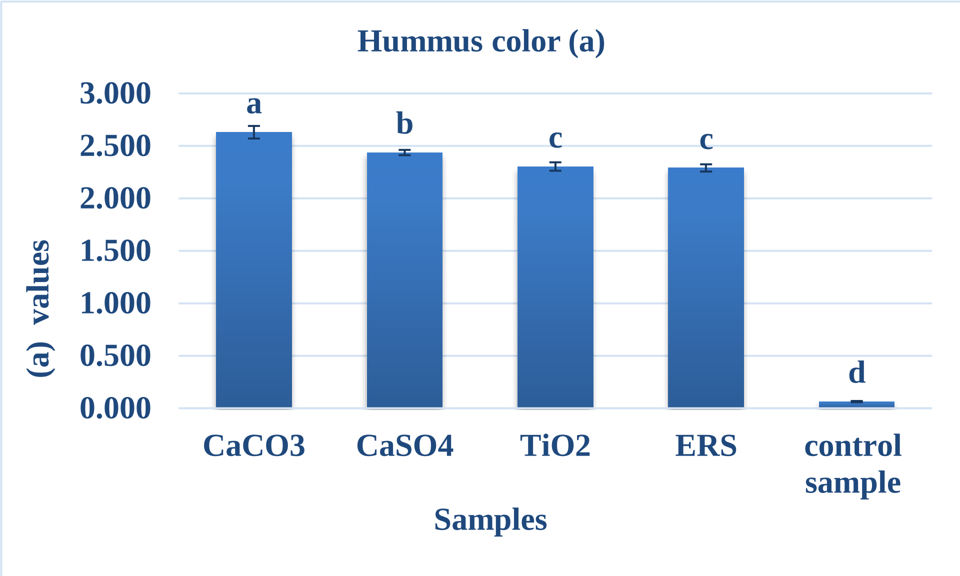


Figure 4.9 Color value (a) yellow color of hummus with 5 whitening agents

4.2.7. The effect of adding whitening agents on the sensory properties of hummus

The effect of adding whitening agents on the sensory properties of the products was studied using a sensory panel, Sensory evaluation of all samples was conducted twice: on day 0 and day 30.

The following attributes were assessed: (acidity, saltiness, bitterness, and mildness of hummus flavor). Samples were also evaluated for their flavor, texture (both creamy and hard), and color (both light and dark). The hummus was evaluated according to how it looked and smelled. (Sahu, 2020) (Morten Meilgaard, 1999).

After comparing all products in terms of light colors on day 0, RS was found to have the lowest sensory rating (Figure (4.10)). The sample contained rice starch and was more or less identical to the titanium dioxide sample. The two samples containing rice starch and titanium dioxide received higher ratings than the calcium sulfate sample, The calcium carbonate sample ranked lowest in terms of light color, compared to other formulas of hummus

During the 30-day sensory evaluation of the sample, a high level of sour taste was observed in all products including RS and samples containing bleaching agents such as rice starch, titanium dioxide, calcium sulfate, and calcium carbonate. In addition, there was a slight increase in hardness in all samples on day 30, which can be attributed to the decrease in moisture content (Table (4.1)). (Gustavo V and Barbosa-Cánovas, 2007). According to Hamza Al-Qadiri (2020), the reason for this increase in acidity is the decrease in pH (table (4.1)) caused by microbial development throughout the storage period. However, during the evaluation period, there were no noticeable changes in the appearance, color, or smell of the hummus.

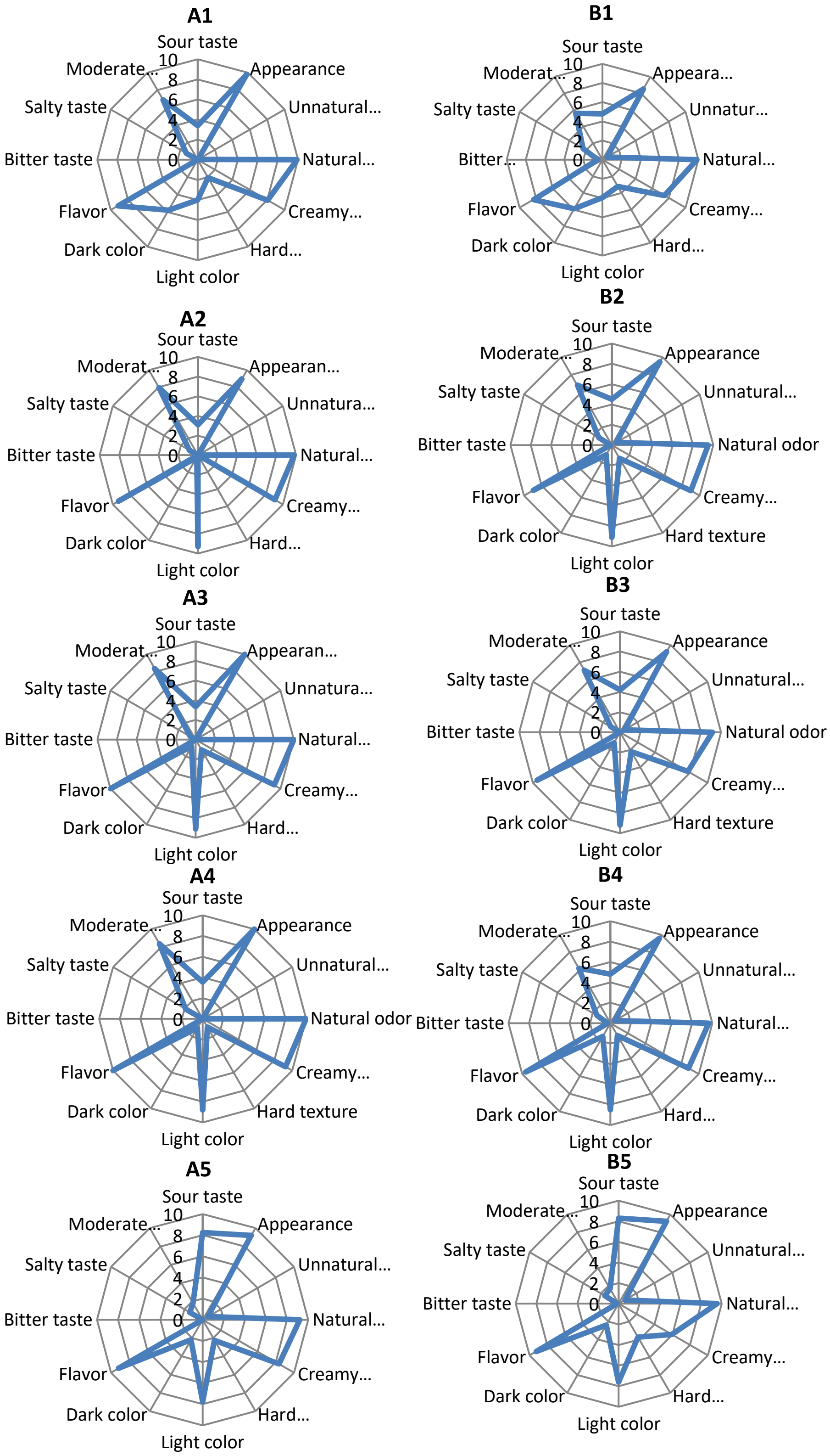


Figure 4.10 Sensory evaluation of hummus samples on day 0 (A), and day 30 (B) it contains a RS (1) Rice starch (2) Titanium dioxide (3) Calcium sulfate (4) Calcium carbonate (5)

4.3. Palestinian hamam bread

4.3.1. Microbial analysis:

No microbial contamination (TPC, TC, FC, Y&M) was found in the bread samples.

4.3.2. The effect of adding whitening agents on the physical and chemical properties of Palestinian hamam bread:

4.3.2.1. Moisture content and total solid:

The percentages of moisture content and total solids varied significantly for all samples from day 0 to day 7 (including the RS and the samples with whitening agents), as table (4.3) illustrates. The staling process, which modifies the starch granules' structure and reorganizes the moisture in the bread, could be responsible for this variation. (Fadda *et al.* 2014)

Table 4.3 Results of physical tests for hamam bread from day 0 to day 7

	M.C%	**T.S%**	**pH**
Control in day 0	42.32±0.06 a	57.67±0.06 b	5.51±0.01 a
Control in day 7	39.4±0.36 b	60.6±0.36 a	5.41±0.005 b
R.S in day 0	41.83±0.057 a	58.16±0.05 b	5.64∓0.02 a
R.S in day 7	39.83±0.057 b	60.16±0.05 a	5.44∓0.01 b
TiO_2 in day 0	42.26±0.15 a	57.73∓0.15 a	5.78∓0.0058 a
TiO_2 in day 7	38.3±0.1 a	61.7 ∓0.1 a	5.51∓0.0058 b
$CaSO_4$ in day 0	41.61±0.01 a	58.39∓0.01 b	5.716∓0.005 a
$CaSO_4$ in day 7	39.13±0.05 b	60.86∓0.05 a	5.51∓0.0058 b
$CaCO_3$ in day 0	41.56±0.005 a	58.43∓0.005 b	5.74∓0.005 a
$CaCO_3$ in day 7	38.13±0.057 b	61.87∓0.057 a	5.51∓ 0.0058 b

4.3.2.2. Moisture content:

The moisture content results were for control. When compared to TiO_2, no significant difference appeared between them (Figure 4.11). However, when compared to R.S, there is a noticeable difference in moisture content between the two samples. $CaSO_4$ had the lowest moisture content among the samples (control, R.S, and TiO_2). Finally, $CaCO_3$ has the lowest moisture content, as shown in Figure 4.11. These results can be explained by the fact that the reference sample does not contain any additives, which leads to a high moisture content. The remaining samples contain bleaching materials in varying quantities according to the required standards and the Codex Alimentarius. This means that adding whitening agents (ERS, $CaSO_4$, $CaCO_3$) reduces the moisture content of bread.

4.3.2.3. Total solid:

The total solids result in control was lower compared to TiO_2, but no significant difference was found between them. However, when comparing control with R.S, a clear and significant difference in total solids was observed between the two formulas. $CaSO_4$ contains the highest percentage of total solids among the samples (control, R.S, and 3). Finally, TiO_2 exhibits the highest percentage of total solids, as illustrated in Figure (4.11). The previous results can be explained by the fact that the control does not contain any additives, leading to a low percentage of solids. The samples contain varying quantities of whitening agent materials according to the required standards and the Codex Alimentarius. This implies that adding whitening agents (ERS, $CaSO_4$, $CaCO_3$) increases the total solids in the bread.

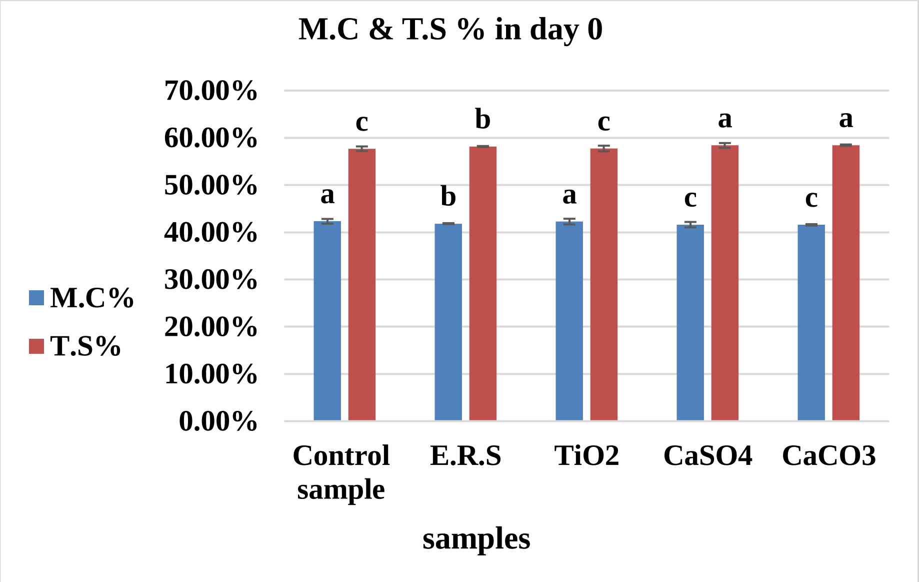


Figure 4.11 Results of physical properties of bread) totals solid and moisture content) at day 0

4.3.3. The effect of adding whitening agents on the physical properties of Palestinian hamam bread in day 7.

4.3.4. Moisture content and total solid:

The moisture content and total solids of the different samples were the same on the seventh day of storage because of the retrogradation. Figure (4.12). There was no significant change between the first and seventh days (Fadda, *et al.* 2014).

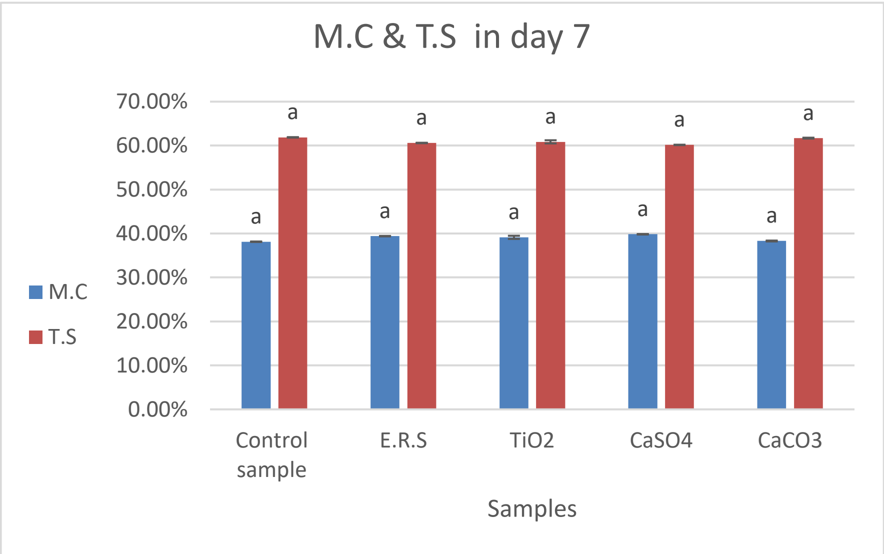


Figure 4.12 Results of moisture content & total solid of Palestinian hamam bread on day 7

Effect of adding whitening factors on the Palestinian hamam bread color

There was no change in color in all samples, whether the RS sample or samples that contain whitening agent (rice starch, titanium dioxide, calcium sulfate, and calcium carbonate) as indicated in Figures (4.13,4.14,4.15)

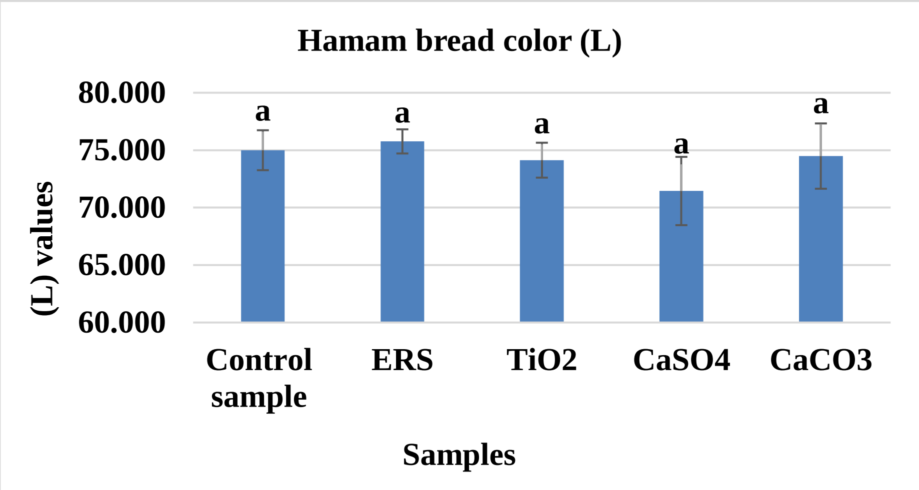


Figure 4.13 Color value (L) white color of Hamam bread with 5 whitening agents

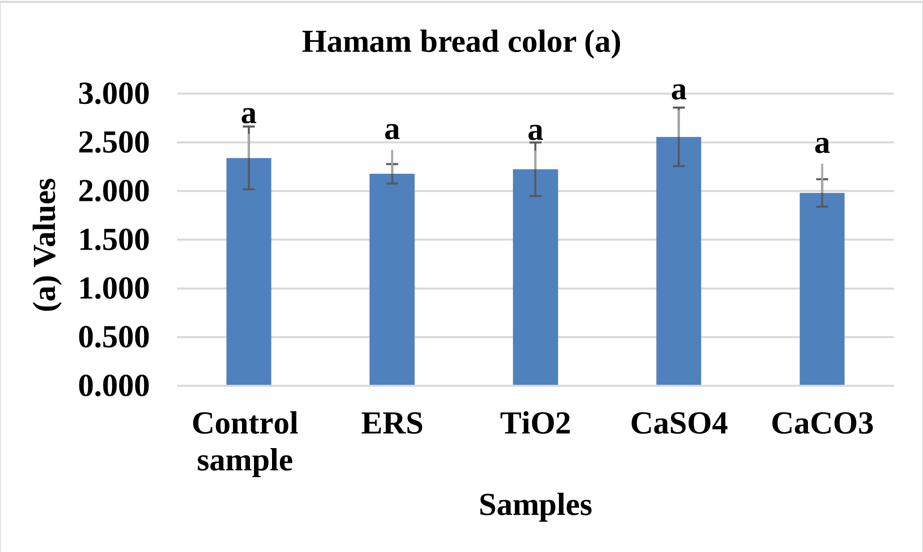


Figure 4.14 Color value (a) white color of Hamam bread with 5 whitening agents

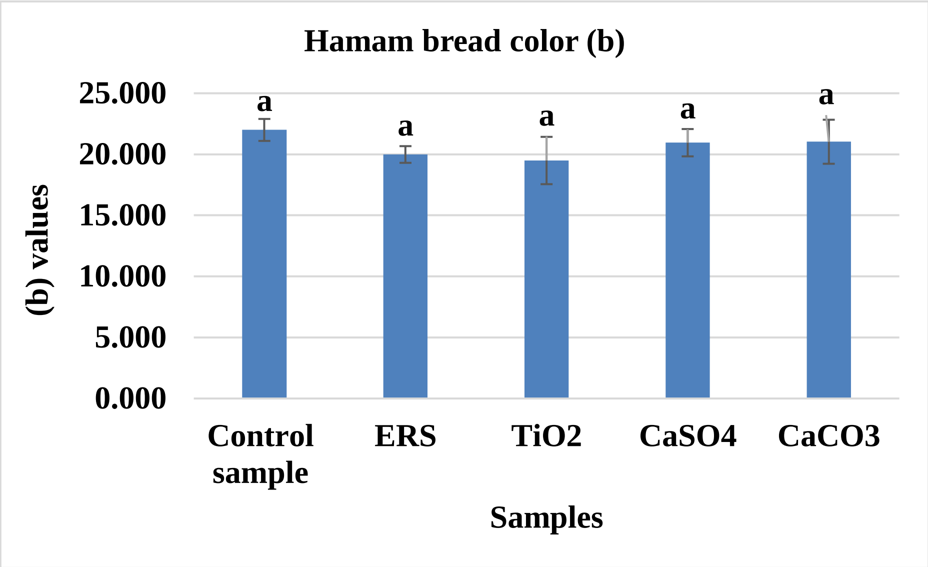


Figure 4.15 Color value (b) white color of Hamam bread with 5 whitening agents

The effect of adding whitening agents on the sensory properties of bread

Sensory evaluation of all samples was performed twice: on day 0 and day 7.

The taste of the bread was evaluated based on saltiness and mildness. In addition, flavor, color (dark and light), and texture (firmness and spongy texture) were evaluated. Palestinian hamam bread was also evaluated based on its aroma and appearance. Figure (4.16)

All samples, including RS and samples containing whitening agents such ERS, titanium dioxide, calcium sulfate, and calcium carbonate, showed a minor increase in product hardness during the 7-day sensory evaluation. Figure (4.16) This result can be related to the decrease in humidity content. Solidification is just one of the many causes of this drop in moisture content.

In contrast to samples containing whitening agents, the RS received the lowest sensory evaluation score, followed by calcium sulfate, calcium carbonate, titanium dioxide, and rice starch. Finally, the two samples containing titanium dioxide and ERS received the highest rating. This was determined by comparing the light color of all products. Figure (4.16)

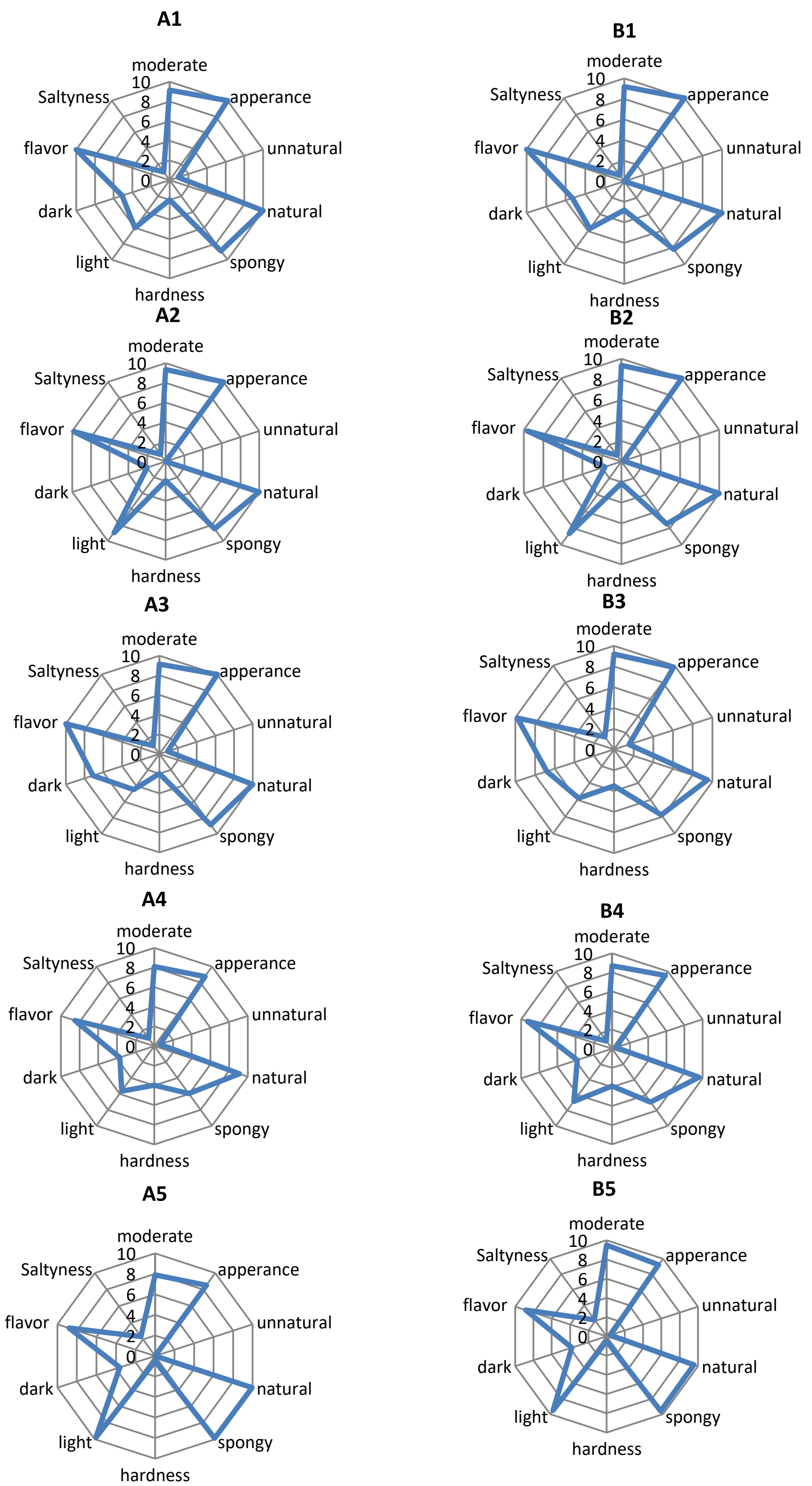


Figure 4.16. sensory evaluation of Palestinian hamam bread samples on day 0 (A), and day 30 (B) it contains an RS (1) Rice starch (2) Titanium dioxide (3) Calcium sulfate (4) Calcium carbonate (5)

4.4. Roasted pumpkin seeds

4.4.1. The effect of adding whitening agents to the physical properties of pumpkin seeds

4.4.1.1. Moisture content and total solid

It was noted that as pumpkin seeds are stored, their moisture content rises and their solid matter content falls. as table (4.4) illustrates. It is clear from this that moisture may readily penetrate dry goods, changing the moisture content and water activity of the tissues from which samples were taken while they were being stored. (Ferreira, *et al*, 2019)

Table 4.4 Results of physical tests for roasted pumpkin seeds from day 0 to day 30.

	M.C (%)	**T.S(%)**
Control	2.13±0.11 d	97.866±0.11 a
control after 10 days	2.33±0.15 c	97.66±0.152 b
control after 20 days	2.53±0.05 b	97.466±0.05 c
control after 30 days	2.86±0.05 a	97.133±0.05 d
TiO_2	3.66±0.05 b	96.93±0.057 a
TiO_2after 10 days	3.36±0.15 a	96.64±0.153 b
TiO_2after 20 days	3.46±0.15 a	96.53±0.153 b
TiO2 after 30 days	3.56±0.11 a	96.433±115 b
E.R. S	2.4±0.1 b	97.5±0.1 a
E.R.S after 10 days	2.7±0.1 a	2.7 ±0.1 a
E.R.S after 20 days	2.8±0.15 a	97.1±0.1 a
E.R.S after 30 days	2.9±0.058 a	97.03±0.05 b
$CaSO_4$	2.28±0.046 a	97.71±0.046 a
$CaSO_4$ after 10 days	2.67±0.115 a	97.33±0.115 a
$CaSO_4$ after 20 days	2.83±0.058 a	97.16±0.059 a
$CaSO_4$ after 30 days	2.83±0.057 a	97.16±0.059 a
$CaCO_3$	2.43±0.057 b	97.567±0.05 a
$CaCO_3$ after 10 days	2.83±0.05 a,b	97.17±0.05 a,b
$CaCO_3$ after 20 days	2.867±0.05 a	97.133±0.05 b
$CaCO_3$ after 30 days	2.9 a	97.1 b

4.4.2. Physical characteristics of roasted pumpkin seeds in day 0

There was a similarity in all results of the moisture content and the total solid in all tested samples as shown in Figure (4.17)

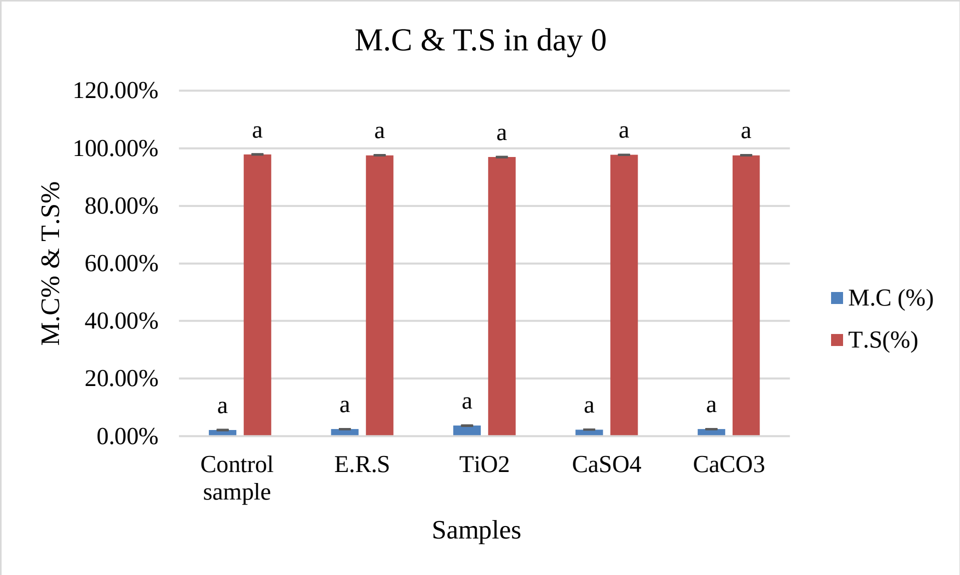


Figure 4.17 Results of physical characteristics of samples at day 0

4.4.3. Physical characteristics of roasted pumpkin seeds in day 30

There were no significant differences among all samples regarding the moisture content and the total solid as shown in Figure (4.18)

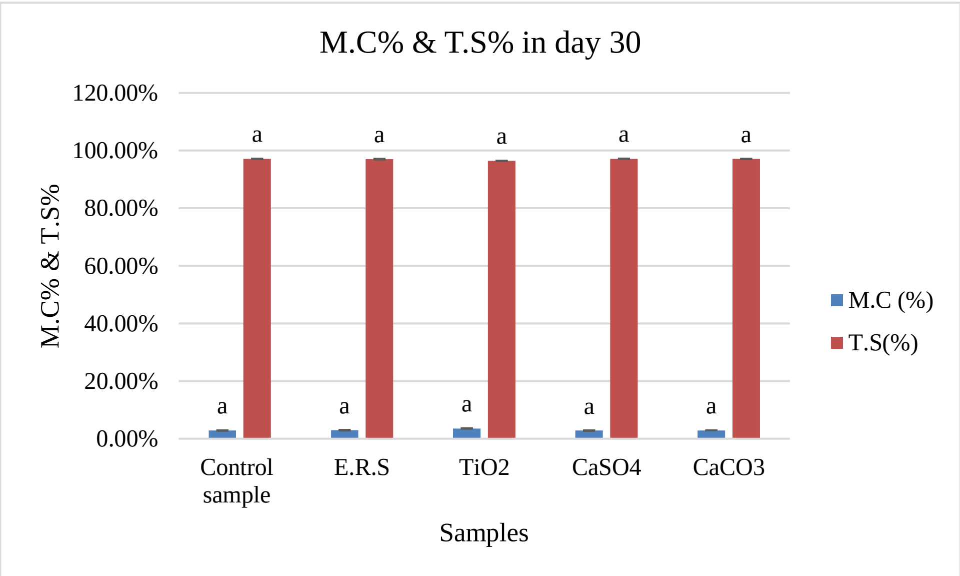


Figure 4.18 Results of physical tests of pumpkin seeds in day 30

4.4.4. The effect of adding whitening agents on the color of pumpkin seeds

It was observed that there was a significant difference in the degree of whiteness between the formulas. The formulas (control and TiO_2) had the highest values and were evenly white, which was significantly higher than the control. $CaSO_4$ was less white than R.S and TiO_2. Finally, $CaCO_3$ was significantly less white than R.S, TiO_2, and CaSo4 and closer in color to control. These observations were supported by the results shown in Figure (4.19).

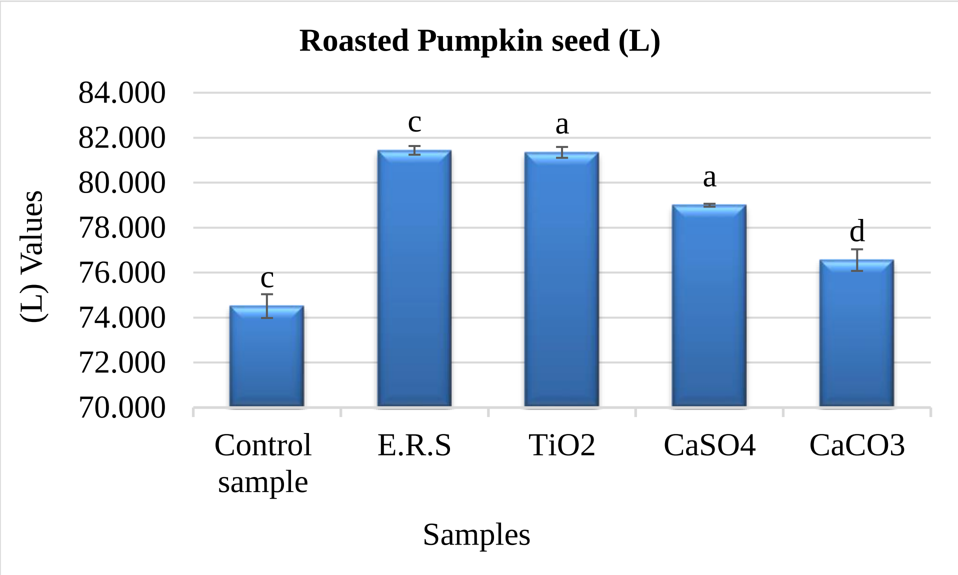
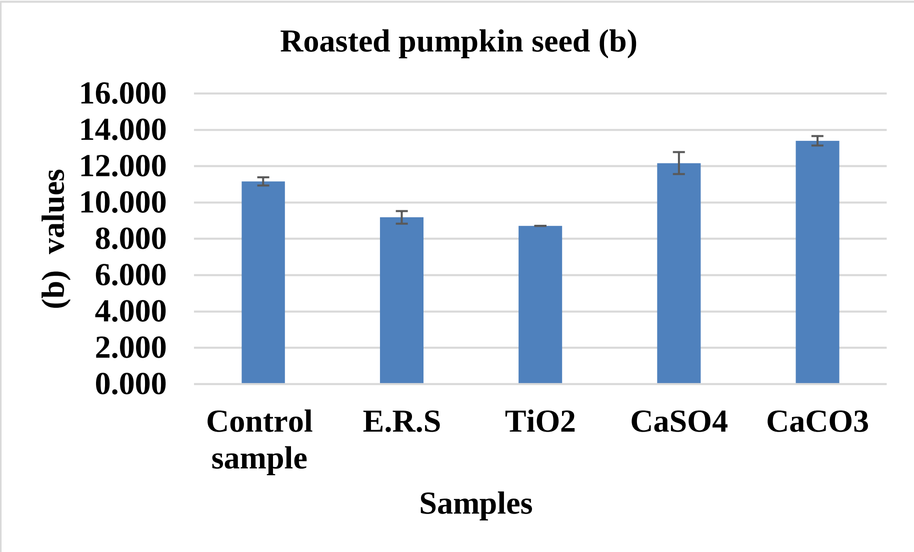


Figure 4.19 Color value (L) white color of pumpkin seeds with 5 whitening agents

The results of the experiment showed that the samples that contained $CaSO_4$ and $CaCO_3$ were noticeably more yellow than the RS. Conversely, it was discovered that the samples with TiO_2 and R.S were the least yellow of all the samples. The findings depicted in figure (4.20) corroborated these findings.



It was found that $CaCO_3$ had the highest level of redness, followed by control. The red color of $CaSO_4$ was significantly different from that of control. Additionally, R.S and TiO_2 had significantly lower levels of redness than the other formulas. These results are illustrated in Figure 4.21)

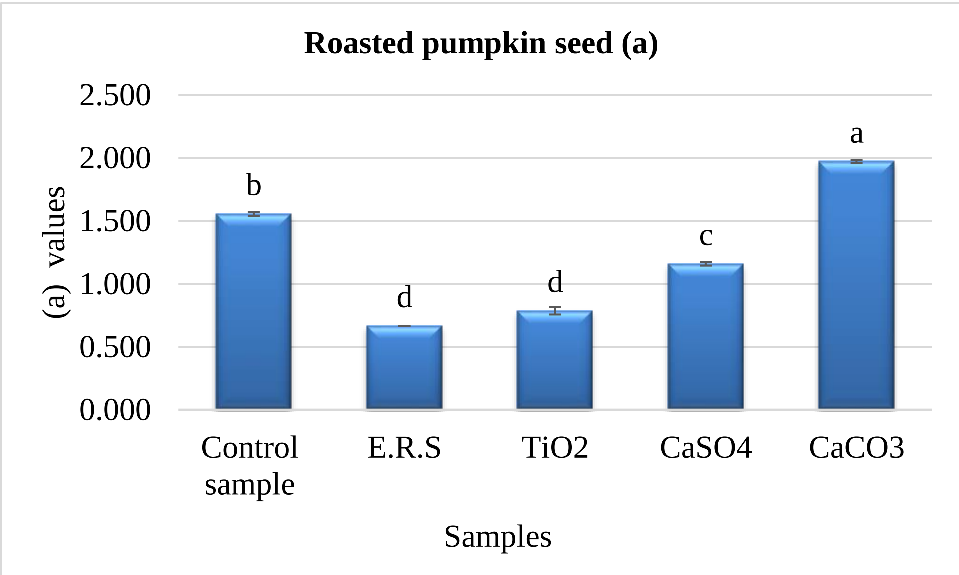


Figure 4.21 Color value (a) white color of pumpkin seeds with 5 whitening agents

4.4.5. The effect of adding whitening agents on the sensory properties of roasted pumpkin seed

Sensory evaluation of all samples was performed twice: on day 0 and day 30.

The taste of the pumpkin was evaluated based on acidity, saltiness, bitterness and mildness. In addition, flavor, color (dark and light) and texture (moist and crunchy) were evaluated. Pumpkin seed were also evaluated based on their aroma and appearance.

The products were found to have a consistently crunchy feel over the course of the 30-day sensory evaluation, with the exception of the two samples that contained calcium carbonate and titanium dioxide, where the crunchiness somewhat diminished. On the other hand, the remaining products retained their original color, flavor, odor, texture, and look/appearance.

Regarding light color, all products were ranked from lowest to highest in terms of sensory evaluation. The samples containing calcium carbonate, calcium sulfate, and rice starch were found to have the highest sensory evaluation score, while the RS received the lowest evaluation. Figure (4.22)

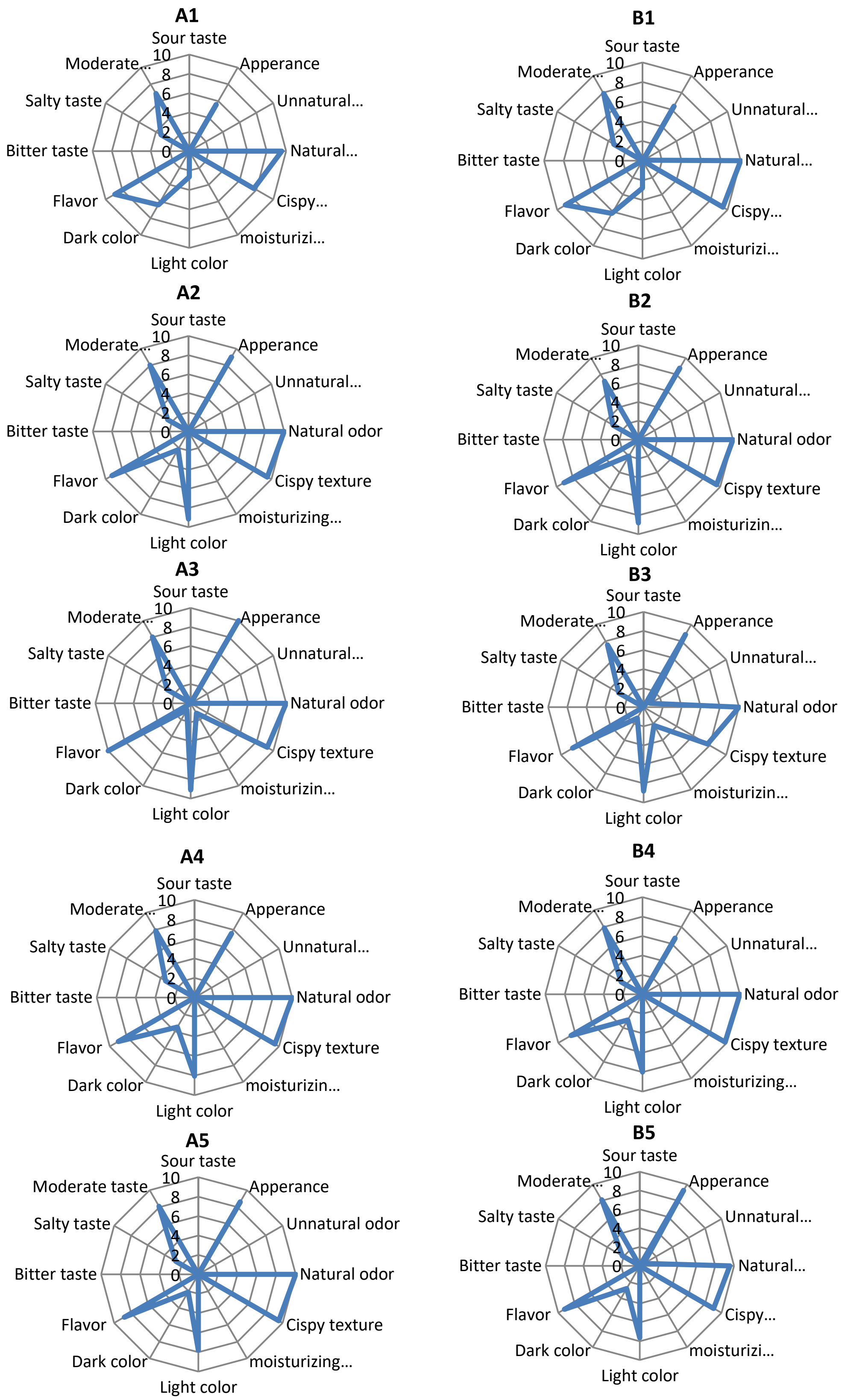


Figure 4.22 Sensory evaluation of Pumpkin samples on day 0 (A), and day 30 (B) contains an RS (1) Rice starch (2) Titanium dioxide (3) Calcium sulfate (4) Calcium carbonate (5)

Chapter five: Conclusion and Recommendations

Due to safety concerns, titanium dioxide use has recently been prohibited (EFSA Panel on Food Additives and Flavorings (FAF), 2021, p. 1). The demand for substitute materials that can produce a comparable degree of whiteness resulted. The three substitutes for titanium dioxide that were evaluated in this study were calcium sulfate, calcium carbonate, and rice starch.

The results reveal that among the potential substitutes for titanium dioxide in hummus, rice starch appears as the closest alternative, showing comparable properties during CIE-LAB analysis and sensory evaluation. Despite the high solid content, rice starch maintains a close resemblance to titanium dioxide in terms of its sensory attributes and color properties as evaluated by the CIE-LAB methodology. These findings support the viability of rice starch as a reliable substitute for TiO_2 in hummus formulations, offering important information for industrial and scientific decision-making in the development of food products.

In the analysis of bread samples using color space (CIE - LAB), no clear discrepancies were observed between the alternatives. Notably, sensory evaluation unequivocally favored rice starch as the optimal alternative.

When it came to roasted pumpkin seeds, rice starch was the best substitute. It showed a similar level of whiteness in both the sensory assessment and color tests. Furthermore, no significant differences were seen in the moisture content or total solids content, indicating that rice starch was an appropriate selection for this use.

This study highlights the necessity for more investigation into titanium dioxide substitutes. Comprehensive studies into how these approaches affect various aspects outside the scope of this study should be prioritized. To fully comprehend these alternatives' efficacy and possible uses, it is also necessary to look at different concentrations of these alternatives. The advancement of scientific knowledge and the useful application of alternative materials in pertinent industrial processes depend heavily on the need for ongoing study.

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تقييم استبدال ثاني أكسيد التيتانيوم بعوامل التبييض الطبيعية في المنتجات الغذائية وتأثيره على الجودة واستقرار التخزين

اسم الطالبة: هديل عماد محي الدين جابري

اسم المشرف: د.زياد عياد

ملخص:

يبحث هذا البحث في عوامل التبييض المختلفة التي يمكن استخدامها في المنتجات الغذائية كبديل لثاني أكسيد التيتانيوم، والذي تم حظره بسبب مخاوف تتعلق بالسلامة. الغرض من الدراسة هو إيجاد بديل يمكن أن ينتج نفس التأثير اللوني لثاني أكسيد التيتانيوم، ويكون ثابتًا أثناء التخزين، ولا يتأثر بالحرارة أو الحموضة، ولا يغير الخصائص الفيزيائية والكيميائية والحسية للمنتج.

تم اختبار العديد من المواد الغذائية بحثًا عن بدائل لثاني أكسيد التيتانيوم، مثل خبز الفينو، وبذور اليقطين المحمصة، والحمص، باستخدام كبريتات الكالسيوم، وكربونات الكالسيوم، ونشا الأرز كبدائل. كربونات الكالسيوم هي مادة طبيعية توجد عادةً في الصخور، ويمكن التعرف عليها من خلال شكل مسحوق أبيض ناصع. وكثيرا ما يستخدم في الطبخ كمقوي للطعام وعامل تبييض. من ناحية أخرى، يعتبر النشا مكونًا شائعًا موجودًا في الأرز. عند استخلاصه من السويداء من نواة الأرز، فإنه يظهر كمسحوق أبيض عديم الرائحة، عديم النكهة، يتميز بوجود حبيبات مجهرية ، والتي تساهم في مجموعة واسعة من التطبيقات في عمليات الطبخ المختلفة والتركيبات. كما تُستخدم كبريتات الكالسيوم بشكل شائع كعامل تبييض مناسب ويُسمح باستخدامها في تصنيع الأغذية، على الرغم من انخفاض قابليتها للذوبان في الماء.

درست الدراسة بشكل منهجي تأثير عوامل التبييض على الخصائص الفيزيائية والميكروبية والكيميائية واللونية والحسية ومحتوى الرطوبة والمواد الصلبة الإجمالية للمنتجات الغذائية خلال فترة التخزين الموصى بها. تم إجراء تقييمات حسية على مواد غذائية مختلفة لتقييم نكهتها ولونها وطعمها ومظهرها ورائحتها وملمسها. ولوحظت أفضل النتائج في المنتجات التي تحتوي على نشا الأرز، تليها كبريتات الكالسيوم وكربونات الكالسيوم. كان أداء نشا الأرز أفضل في مركبات الحمص منه في بدائل بذور اليقطين كما هو محدد باستخدام تقنية مساحة الألوان CIE-LAB. على الرغم من عدم العثور على بقايا

عامل تبييض يمكن اكتشافها في خبز Fino بعد تحليل CIE-LAB، فقد تم تحديد نشا الأرز ليكون البديل الأفضل بناءً على التحليل الحسي.

يسلط هذا التحقيق الشامل الضوء على جدوى استخدام عوامل التبييض في تعزيز أو الحفاظ على سمات الجودة لمختلف المنتجات الغذائية مع الالتزام بأنظمة السلامة التي وضعتها وكالات تنظيم الأغذية الوطنية.

Appendix 1:

1.1. Collect samples and prepare them for testing.
1.2. Chemical analysis.

1.2.1. Moisture content % determination.
1.2.2. Determination of crude fat content.
1.2.3. Determination of protein content.
1.2.4. Determination of ash contents.

1.3. Color measurement.
1.4. Microbial tests.
1.5. Sensory evaluation.

Appendix 1:

1.1.Collect samples and prepare them for testing

After preparing the formulations in the laboratory, samples were collected for testing by obtaining three samples of each formulation into sterile containers. The samples were examined for sensory, microbial and color characteristics as is, while chemical tests were carried out in ground form.

The coding of the samples was as follows:

Table 1.1 The coding of the samples.

Hummus	**Fino bread**	**Roasted pumpkin seeds**
H.F 1 A	B.F 1 A	P.F 1 A
H.F 1 B	B.F 1 B	P.F 1 B
H.F 1 C	B.F 1 C	P.F 1 C
H.F 2 A	B.F 2 A	P.F 2 A
H.F 2 B	B.F 2 B	P.F 2 B
H.F 2 C	B.F 2 C	P.F 2 C
H.F 3 A	B.F 3 A	P.F 3 A
H.F 3 B	B.F 3 B	P.F 3 B
H.F 3 C	B.F 3 C	P.F 3 C
H.F 4 A	B.F 4 A	P.F 4 A
H.F 4 B	B.F 4 B	P.F 4 B
H.F 4 C	B.F 4 C	P.F 4 C
H.F 5 A	B.F 5 A	P.F 5 A
H.F 5 B	B.F 5 B	P.F 5 B
H.F 5 C	B.F 5 C	P.F 5 C

1.2.Chemical analysis

(Moisture content%, Fat%, Protein%, Fiber%, Ash%)

1.2.1. Moisture content%:

Materials and tools:

- Samples
- Moisture analyzer

Method:

The device was whistled, and 2 grams of samples were weighed. After that, the device was closed, and it waited several minutes to give the result. (Rossa, et al, 2015)

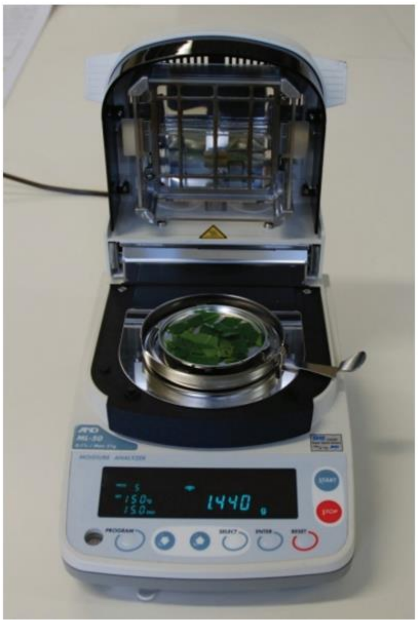

Figure 1.1 Moisture Analyzer

1.2.2. Determination of crude fat content:

1.2.2.1. Materials:

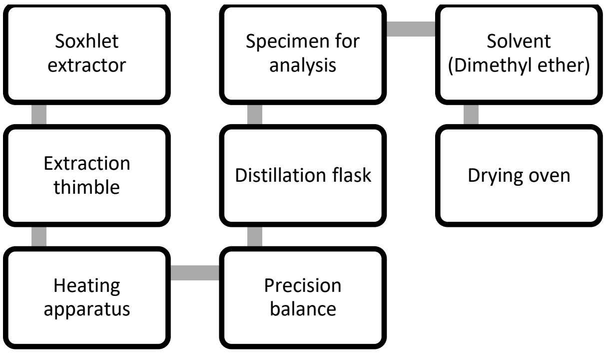

Figure 1.2 Materials used in fat content test.

1.2.2.2. Procedure:

1.2.2.2.1. Weigh the extraction thimble and document the measurement as W1

1.2.2.2.2. Insert the specimen into the thimble and record the total weight as W2

1.2.2.2.3. Place the thimble with the specimen into the Soxhlet extractor.

1.2.2.2.4. Add the solvent to the distillation flask to commence the extraction process.

1.2.2.2.5. The solvent vapor rises to the condenser, where it condenses and then drips onto the specimen in the thimble, facilitating the extraction of fat.

1.2.2.2.6. Gather both the solvent and the extracted fat in the distillation flask.

1.2.2.2.7. Continue the procedure until a substantial amount of fat is extracted from the specimen.

1.2.2.2.8. The Soxhlet process includes the vaporization of the solvent, its condensation, and the subsequent dripping onto the specimen.

1.2.2.2.9. Following the extraction, take out the thimble and evaporate the solvent from the collected fat in the distillation flask.

1.2.2.2.10. Measure the weight of the extraction thimble along with the remaining residue and record this value as W3

1.2.2.2.11. Use the provided formula to determine the fat content.

$$Crude\ fat\ content\ (\%) = \frac{(w2 - (\ w3 - w1))}{w2} \times 100$$

1.2.3. Determination of crude protein content

1.2.3.1. Materials:

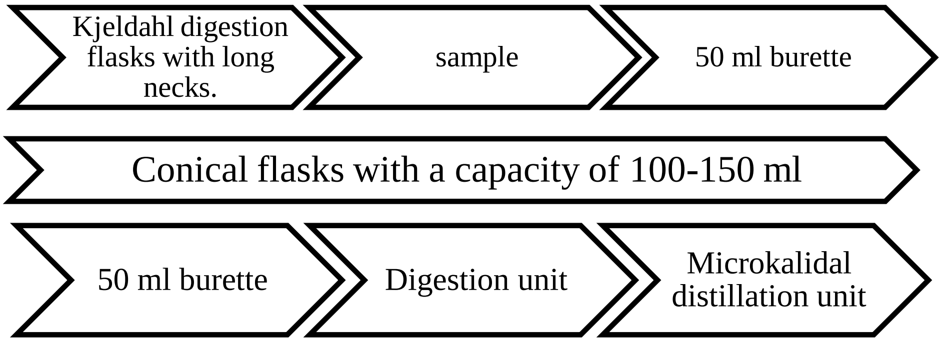

Figure 1.3 Materials and toles for Kjeldahl method

1.2.3.2. Procedure:

The Kjeldahl method described in AOAC (1990) was used to determine the protein content. Approximately 0.5 g of finely ground samples are weighed into a digestion tube during this procedure. Boiling flakes are added to the digestive tube together with a catalyst (3.5 g of K2SO4 and 3.5 mg of selenium; Gerhardt, Germany). The samples are heated gradually (by 50 °C/15 min) from 80 to 420 °C over a 3-hour digesting process at 420 °C. An additional hour of digestion is done in cases where the sample is opaque. After processing is finished, the sample is left to cool to room temperature. Then, a 250 mL beaker with 30 mL of boric acid is placed next to the digesting tube in a distillation unit (Gerhardt, Germany). Titration is performed on the resultant solution using 0.1 M HCl.

$$protein\ content\ (\%) = \frac{[(ml\ of\ HCl - ml\ of\ blank) \times N\ of\ HCl\ \times 1.4007]}{weight\ of\ sample\ (g)}$$

1.2.4. Ash contents Determination

The crucibles [w1] were initially weighed. About 1 gram of samples was meticulously measured and placed into crucibles [w2], which were subsequently exposed to an oven for 24 hours at 525°C. Following this process, the crucibles were retained in the furnace until the temperature descended to 250°C, after which they were allowed to cool to room temperature and then reweighed [w3]. The ash content was ascertained through the application of a specific calculation.

$$\text{ash content (\%)} = \frac{(w3 - w1)}{w2} \times 100$$

1.3. Color analysis:

The CIE-LAB system was used. The HunterLAB device was utilized to examine three samples of each composition. This involved placing the device on the sample and then pressing the control button, after which the results appeared. The system uses the values of L*, a*, and b* to determine the position of the sample on the color axis. The L* value indicates how light or dark it is, while the a* value indicates green with negative values and red with positive values. On the other hand, b* indicates blue with negative values and yellow with positive values.

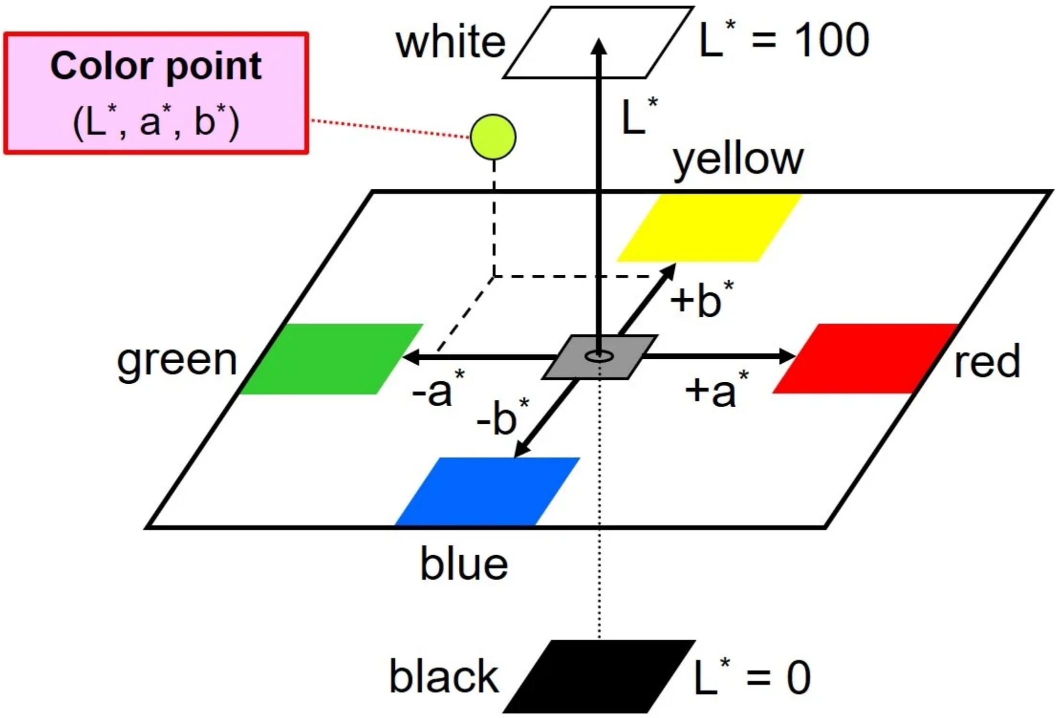

figure 1.4 An illustration of the L, a, b (CIE Lab) system in three dimensions

1.4. Microbial tests:

1.4.1. We prepare the Petri dishes by writing the date, sample code, and dilution name of the assay (TPC /Total Coliform /fecal coliform/E.coli/yeast and mold).

1.4.2. Take 10 g of the sample to be examined and put it with 90 g of peptone water and put it in a stomacher bag for two minutes.

1.4.3. Take 1 ml of the sample through a micro pipet and put it inside the petri dish.

1.4.4. Take 1 ml of the sample through a micro pipet and put it inside the petri dish.

1.4.5. Take 1 ml of the sample in peptone water to make a dilution.

1.4.6. Take 1 ml of it and put it in the petri dish and then take 1 ml and put it in peptone water to make a dilution.

1.4.7. The dishes are placed in the incubators as follows:

- 24 hours for the Total Coliform at a temperature of 37°C.
- 48 hours for the TPC at a temperature of 37°C.
- 72 hours for the yeast and mold at a temperature of 25°C.
- 24 hours for the Fecal coliform at a temperature of 45°C.
- 24 hours for the E. coli at a temperature of 45°C.

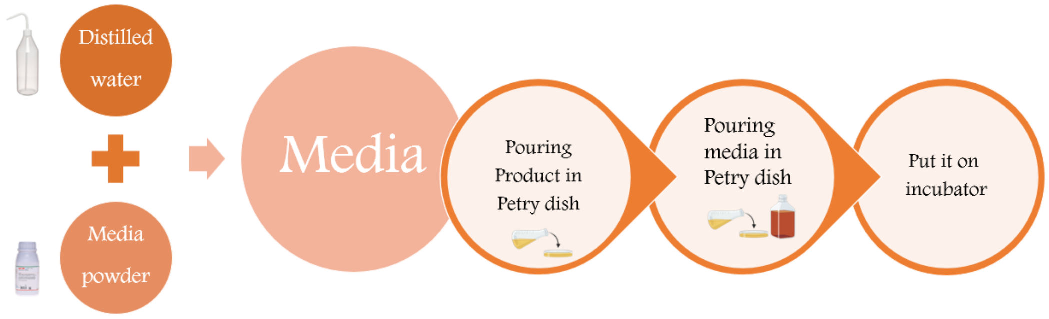

Figure 1.5 Method of media preparation.

1.5. Sensory evaluation:

Eight expert individuals were selected to perform the sensory evaluation. Appropriate product containers were used to store samples; For example, BET plastic bags have been used for bread and pumpkin seeds, and BET plastic containers have been used for hummus.

All food samples were evaluated (Food questionnaire), including the reference sample (RS) and samples containing bleaching agents. The following characteristics of chickpeas were studied: texture (creamy or firm), color (light or dark), odor (natural or abnormal), and appearance (sour, mild, salty, or bitter). Figure (1.8) Pumpkin seeds' texture (moist or crunchy), color (light or dark), scent (natural or artificial), and appearance were taken into account. (Figure 1.7) Finally, the characteristics of the bread: texture (hard or spongy), color (light or dark), shape, and taste (mild, salty). (Figure 1.6) (Inaya M. Hassan, 2020)

Bread questionnaire

جامعة القدس
Al-Quds University

Sample Code: ____________

SENSOR EVALUATION

TASTE — المذاق

MODERATE ______________________ معتدل

SALTYNESS ______________________ مالح

FLAVOR — النكهة

____

COLOR — اللون

DARK ______________________ غامق

LIGHT ______________________ فاتح

TEXTURE — القوام

HARD ______________________ متصلب

SPONGY ______________________ أسفنجي

ODOR — الرائحة

NATURAL ______________________ طبيعية

UN NATRUAL ______________________ غير طبيعية

APPEREANCE — المظهر

____

Food Connoisseur Sign توقيع متذوق الطعام

____

Figure (1.6) Bread questionnaire.

Roasted Pumpkin seeds questionnaire

جامعة القدس
Al-Quds University

Sample Code: ____________

SENSOR EVALUATION

TASTE المذاق

SOUR ____________ حامض

MODERATE ____________ معتدل

SALTYNESS ____________ مالح

BITTERNESS ____________ مر

FLAVOR النكهة

____

COLOR اللون

DARK ____________ غامق

LIGHT ____________ فاتح

TEXTURE القوام

MOISTURIZING TEXTURE ____________ قوام رطب

CRISPY TEXTURE ____________ قوام مقرمش

ODOR الرائحة

NATURAL ____________ طبيعية

UN NATRUAL ____________ غير طبيعية

APPEREANCE المظهر

____

Food Connoisseur Sign توقيع متذوق الطعام

____

Figure (1.7) Roasted pumpkin seeds questionnaire.

Hummus questionnaire

جامعة القدس
Al-Quds University

Sample Code: ____________

SENSOR EVALUATION

TASTE المذاق

SOUR ________________ حامض

MODERATE ________________ معتدل

SALTYNESS ________________ مالح

BITTERNESS ________________ مر

FLAVOR النكهة

____

COLOR اللون

DARK ________________ غامق

LIGHT ________________ فاتح

TEXTURE القوام

HARDNESS ________________ صلابة

CREAMY ________________ كريمي

ODOR الرائحة

NATURAL ________________ طبيعية

UN NATRUAL ________________ غير طبيعية

APPEREANCE المظهر

____

Food Connoisseur Sign توقيع متذوق الطعام

____

Figure (1.8) Hummus questionnaire.