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Abstract

Background: Rice gluten-free products, are perfectly suitable for all patients who are warned against consuming gluten, but these products lack important nutrients that may cause problems in body health. Therefore, milk thistle flour(MTF) is superior source for increasing the nutritional value of these products.

Methods: Proximate analysis, Mineral analysis, the nutritional profile of the biscuits, Antioxidant evaluation, Texture profile analysis, Color parameters and sensory evaluation were determined.

Results: Proximate analysis revealed that milk thistle flour contains significantly higher levels of protein, ash, fat, and dietary fiber compared to rice flour, with protein and iron contents being over three and seven times greater, respectively. Mineral analysis showed milk thistle's superior content of calcium, potassium, magnesium, and phosphorus. Substitution with MTF led to notable improvements in the nutritional profile of the biscuits, including increased protein (from 6.73% to 7.91%) and fiber (from 2.32% to 2.90%). Antioxidant evaluation showed significantly higher total phenolic and flavonoid contents in milk thistle, alongside stronger DPPH scavenging activity and lower IC₅₀ values compared to rice flour. Texture profile analysis demonstrated that higher MTF inclusion increased hardness while decreasing fracturability and adhesiveness. Color parameters (ΔE^* , a^* , b^*) and sensory scores declined with increasing MTF levels, particularly at 20–25% substitution, due to pigmentation and fiber content. However, lower substitution levels (5–10%) maintained favorable acceptability while enhancing nutritional and antioxidant value. These findings will be helpful in increasing the nutritional value of baked goods, especially gluten-free ones.

Keywords: nutritional value, Mineral, Protein, Texture profile, Color parameters

المخلص عربي

الخلفية: تعتبر المنتجات الخالية من الجلوتين المصنوعة من الأرز مناسبة تمامًا لجميع المرضى الذين يُحذرون من تناول الجلوتين، إلا أن هذه المنتجات تقتصر إلى عناصر غذائية مهمة قد تُسبب مشاكل صحية. ويعد دقيق حليب الشوك مصدرا ممتازا لزيادة القيمة الغذائية لهذه المنتجات

الطريقة: تم تحليل التركيب الكيميائي التقريبي للمواد الخام، وتقدير المعادن، وتحديد القيمة الغذائية للبسكويت المستبدل فيه الأرز بدقيق حليب الشوك، والتقييم المضادات للأوكسدة، وتقدير قوام ولون البسكوت والتقييم الحسي.

النتائج: كشفت النتائج عن احتواء دقيق حليب الشوك على مستويات أعلى بكثير من البروتين والرماد والدهون والألياف الغذائية مقارنةً بدقيق الأرز، حيث يزيد محتوى البروتين والحديد بأكثر من ثلاثة أضعاف وسبعة أضعاف على التوالي. أظهر تحليل المعادن محتوى أعلى من الكالسيوم والبوتاسيوم والمغنيسيوم والفوسفور في حليب الشوك. كذلك تحسينات ملحوظة في القيمة الغذائية للبسكويت بما في ذلك زيادة البروتين (من ٦.٧٣ % إلى ٧.٩١ %) والألياف (من ٢.٣٢ % إلى ٢.٩٠ %). ارتفاعاً ملحوظاً في إجمالي محتوى الفينولات الكلية والفلافونويد إلى جانب نشاط أقوى DPPH وانخفاض قيم IC_{50} مقارنةً بدقيق الأرز. ووجد أن بزيادة نسبة الاستبدال بدقيق حليب الشوك تزيد صلابة البسكويت مع تقليل قابلية الكسر والالتصاق كذلك انخفاض معايير اللون ΔE^* ، a^* ، b^* ودرجات التقييم الحسي وخاصةً عند الاستبدال ٢٠ % و ٢٥ %، ومع ذلك، حافظت مستويات الاستبدال المنخفضة (١٠-٥ %) على قبولها الجيد مع تعزيز قيمتها الغذائية ومضادات الأكسدة. هذه النتائج سيكون لها دورا مهما في زيادة القيمة الغذائية للمخبوزات، وخاصةً الخالية من الجلوتين.

الكلمات المفتاحية: القيمة الغذائية، المعادن، البروتين، ملف القوام، معايير اللون

INTRODUCTION

Some conditions that require gluten-free products include dermatitis herpetiformis, Celiac disease, and illnesses including gluten-sensitive ataxia and Non-Celiac Gluten Sensitivity (Al-Toma et al., 2019). Gluten-free products are in high demand among both those obliged to consume them and those seeking a healthy diet. So these products require improvements in ingredients and formulations, as well as producing functional foods (Falguera et al., 2012). Gluten-free diet (GFD) is an imbalanced diet that hides a number of nutritional deficits. It is recognized that gluten-free products have lower nutritional value (Lerner et al., 2019). The fact that most GFD products are not fortified exacerbates the deficits. According to (Melini and Melini 2019) and most studies, GFD is low in proteins, fibers, iron, folate, zinc and potassium, but high in fat, carbohydrate, sugars, and salt.

Rice, brown rice, corn, millet, teff, chickpea, amaranth, quinoa, buckwheat, oat, and tiger nut are types of gluten free flours (Culetu et al., 2021). Rice (*Oryza sativa* L.) is one of the most commonly utilized grains in these gourmet cuisine preparations. Rice flour is a popular component in gluten-free foods due to its high digestible protein content and sensory qualities (Osella et al., 2014). However, replacing wheat flour with rice flour frequently results in a notable decline in the quality of developed goods. Therefore, optimization of rice flour formulas is crucial (Nuno et al., 2011). Rice flour is devoid of gluten, low in protein, fiber, salt, and fat, and high in easily digestible carbs. It is widely used as a wheat substitute in gluten-free products (Korus et al., 2009).

The replacement of wheat flour with rice flour typically results in a notable decline in the quality of developed products. As a result, optimizing rice flour formulations is critical (Nuno et al., 2011). The replacement of rice for gluten-containing prolamins increased the patient's risk of protein, fiber, and folate deficits (Hosseini et al., 2018) as well as a higher glycemic index. This may explain why CD patients with and without treatment have higher rates of metabolic syndrome and cardiovascular morbidity (Lerner and Matthias 2015; Ciccone et al. 2018).

Food makers should pay greater attention to the nutritional value of gluten-free products (**Al-Zaben et al., 2023**), with biscuits being one of the most common. Biscuits are commonly recognized as a snack item among children and adults. They are also regarded an excellent supplemental meal for undernourished children, making them a regular distribution item for developmental organizations (**Kaur et al., 2019 and Poole et al., 2021**)

Milk thistle (*Silybum marianum* L. Gaertn.) is a spiky herb belonging to the Asteraceae family (**Marceddu et al., 2022**). It has been used in medicine for millennia, mostly to treat kidney, spleen, and liver ailments (**Flora et al. 1998**). Milk thistle has anti-inflammatory and antioxidant properties, and has been used to treat Alzheimer's disease, burns, osteoporosis, cholestasis, sepsis, hypercholesterolemia, and diabetes. It can also reduce the side effects of chemotherapy and radiation therapy for cancer (**Devin et al., 2022**). Its antioxidant properties are attributed to its high content.

Milk thistle is a rich source of micronutrients such as minerals, amino acids, fatty acids and phytochemicals exhibiting nutraceutical effects on human health. **This study aimed to Enhance gluten free biscuits with nutrients for people especially with gluten allergies and for improving the physicochemical Properties of the gluten free biscuits.**

MATERIALS and METHODS

MATERIALS:

Egyptian rice flour, obtained from local market. Milk thistle obtained from Haraz Market for Seeds, Spices and Herbs in Bab Al-Khalq, Egypt.

METHODS

Preparation of milk thistle seed flour

According to the method described by (**Atta and Imaizumi, 2002**) milk thistle flour was done as follows:

Tab water followed by distilled water were used to wash the seeds. Using air drying oven to dry the seeds. The laboratory electronic mill (Broun, Model 2001 DL, Germany) was used for grinding the seeds at speed 2 for 3 min to pass through 40 mesh sieve. Using polyethylene bags for storing the powder in the deep freezer at -20°C

Extraction

The dried powder (10.0 g) was extracted in triplicate with Et-OH (96% v/v) at room temperature, under stirring. Aqueous suspension of the concentrated Et-OH extract was evaporated to dryness and used for all investigations (**Dzoyem et al., 2014**).

Chemical Composition of raw materials

According to the method described in the **A.O.A.C (2000)**. Crude protein, Moisture, crude fiber, Fat, and ash contents of MTF and RF were determined.

Determination of minerals content.

Using atomic absorption spectrophotometer (Perkin Elmer Model 4100 ZL) minerals (calcium, potassium, magnesium, phosphorus and Iron) were determined according to the methods described in **A.O.A.O (2005)**

Total phenol content analysis

The total phenolics content (TPC) was determined using Folin-Ciocalteu reagent (**Singleton et al., 1999**). Briefly, an amount of 5 µl of the extract was added to 1.70 ml of

distilled water and 300 µl of Folin-Ciocalteu reagent (previously diluted 3-fold with distilled water). The mixture was allowed to stand for 3 min, then 0.5 ml of Na₂CO₃ (20%, w/v) was added to the mixture. After 1 h in dark at room temperature, the absorbance was measured at 760 nm using (UV-Vis Shimadzu (UV-1601, PC). Results were expressed as gallic acid equivalents (mg of gallic acid/g dry weight).

Total flavonoid content analysis

The content of total flavonoid (TFC) in each extract was evaluated using aluminum trichloride solution (AlCl₃) and the colorimetric technique adopted by Djeridane et al. (2006). An aliquot of the crude extract (500 µl) was combined with 500 µl of 2% AlCl₃. The intensity of the pink hue was then measured at 420 nm using Shimadzu's UV-Vis (UV-1601, PC). After 15 minutes. Rutin was chosen as the standard. The results were represented as mg rutin equivalents per gram of dry weight

Antioxidant activity

DPPH radical assay

The antioxidant activity of the investigated materials was assessed using the scavenging activities of the stable 2,2'-diphenyl-2-picrylhydrazyl (DPPH) radical, as reported by **Malik et al. (2017)**. Various quantities (50, 100, and 150 uL) of prepared samples were combined with 3.95 mL of methanol and 1 mL of a 0.2 mmol methanol solution of DPPH. After 30 minutes of incubation in the dark at room temperature, the absorbance was measured at 517 nm against a blank (methanol) using a Shimadzu UV-Vis (UV-1601, PC). The formula for calculating free radical DPPH inhibition in percent (%) was as follows:

$$\text{Percentage inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100 \quad A$$

control is the absorbance of the control reaction (which contains all reagents except the test component), whereas A sample is the absorbance of the test compound. IC₅₀ values (concentration of sample necessary to scavenge 50% of free radicals) were computed using a regression equation based on sample concentration and percentage inhibition of free radical production, and percentage inhibition DPPH was measured. The synthetic antioxidant Butylated Hydroxytoluene (BHT) and natural L-ascorbic acid were employed as positive controls, and all experiments were performed in triplicate.

Biscuits preparation

The biscuits were produced using the procedures published by **Oyewole et al. (1996)**, with minor modifications. A recipe for biscuits with 100% rice flour, 30% sugar, 20% margarine, 1% sodium chloride, 0.5% sodium bicarbonate, 1% ammonium bicarbonate, 0.39% baking powder, and 16 mL of water. A rice biscuit was used as a control, and free gluten biscuits were made by substituting rice flour with varying amounts of Milk Thistle flour (5, 10, 15, 20, and 25%).

In a Kenwood mixer, combine butter and sugar on medium speed until a light and frothy cream forms. Add fresh egg yolk and continue to beat. Water containing sodium bicarbonate, ammonium bicarbonate, and sodium chloride was added to the aforementioned cream and stirred for 5 minutes at 125 rpm to create a homogenous cream. Finally, the level flour of rice was sieved twice, and baking powder was added and combined for 3 minutes at 60 rpm. Dough was made by adding varied amounts of milk thistle to the mix and then rolled on a flat rolling board. Circular biscuits were cut

and placed on prepared baking pans. Baked in an electric oven (Kumatel, Turkey) at 160°C for 15 minutes. The biscuits were chilled for 30 minutes and then kept in airtight containers for 24 hours before further investigation.

Proximate Chemical Composition of Biscuits

The analyses were carried out according to the Association of Official Analytical Chemists International methods (AOAC, 2006). The Kjeldahl procedure with nitrogen was used to determine the total proteins content (protein conversion factor was 6.25, method number 950.36). The Soxhlet method (method no. 935.38) was used to determine the fats content. The ash content was examined by carbonization (method number 923.03). The content of total carbohydrates is the difference after taking into account the moisture, protein, fat and ash content. The caloric value = (4 × protein) + (9 × fat) + (total carbohydrates) × 4 (FAO, 2002). The final result is the average of three repetitions.

Texture Profile Analysis.

A texture analyzer (BROOKFIELD CT3 TEXTURE ANALYZER Operating Instructions Manual No. M08-372-C0113, Stable Micro Systems, USA) was used to measure the texture profile of biscuits in terms of adhesiveness (mj), fracturability (N) and hardness (N) of the samples using the method described by Bourn (1978). Test Type: Compression, Target=5.0mm, Hold Time=0s, Trigger Load: 5.00 N (Newton), Test Speed= 2.00mm/s, Return Speed= 2mm/s, Number of Cycles: 1.0, Pretest Speed: 2 mm/s. Probe: TA-PFS-C. Fixture: TA-BT-KIT, Load Cell: 10,000g. The trials were carried out at ambient conditions.

Color measurement

The color of the biscuits was measured with a handheld portable colorimeter (CHROMA METER CR-400, Japan). The equipment was calibrated with a white tile and findings were presented in terms of brightness (L^*), redness (a^*), yellowness (b^*), and overall color variation index (ΔE).

Sensory Evaluation

Twenty panelists assessed biscuit samples for organoleptic features such as appearance, color, odor, texture, taste, and overall acceptability using the approach proposed by Larmond (1977). The highest score for each characteristic was (20) degrees.

Statistical analysis

Using one-way ANOVA (Rao and Blane, 1985) to make statistical analysis and present data as means $\pm$ SD.

Results and discussion

Fig. (1) showed significant differences in the results of chemical composition between milk thistle and rice flour. Milk thistle (MT) had significantly greater protein content (22.63 ± 0.45) than rice flour (6.72 ± 0.15). milk thistle is approximately 3 times greater than protein in rice flour. Its protein content is more than 3 times greater than that of rice flour. This is consistent with Al-Snafi's (2016) results, which indicated that milk thistle seeds contain 20-30% protein and are high in important amino acids. According to Juliano (2007), rice flour has lower protein content than refined grains.

Results indicates that Ash in milk thistle is approximately 5 times greater than Ash in rice flour. Milk thistle has a larger ash content (5.46 ± 0.12) than rice flour (1.07 ± 0.03), indicating increased mineral density, especially calcium and iron, as indicated in

our earlier investigation. This is consistent with the findings of **Karkanis et al. (2011)**, who studied medicinal plant mineral profiles.

The remarkable fiber content in milk thistle (18.96 ± 0.87) compared to rice flour ($1.57 \pm 0.08\%$) can be attributed to its intact seed coat. This supports the findings of **Apostol et al. (2017)** showing milk thistle's potential as a functional fiber source, whereas refined rice flour loses most bran fiber during processing (**Saunders, 1990**).

Fat content in milk thistle content (25.71 ± 0.63) higher than rice flour ($1.13 \pm 0.05\%$), indicating its oilseed origin. According to **Khan et al. (2007)** and **Nemati et al., (2017)**, who indicated that milk thistle seeds contain 25-31% oil high in linoleic acid, whereas rice bran oil is normally lost during milling (**Hendawy and Khater 2014**).

The moisture differences (milk thistle: 7.93 ± 0.21 and rice flour: 11.54 ± 0.32). Oil seeds contain high percentages of oils, which are non-polar components that do not easily hold water. **Soltani et al., (2014)** refer to low moisture levels resulted in brittleness, whereas greater moisture levels generated plasticizing effects, resulting in lower compression and poor oil recovery.

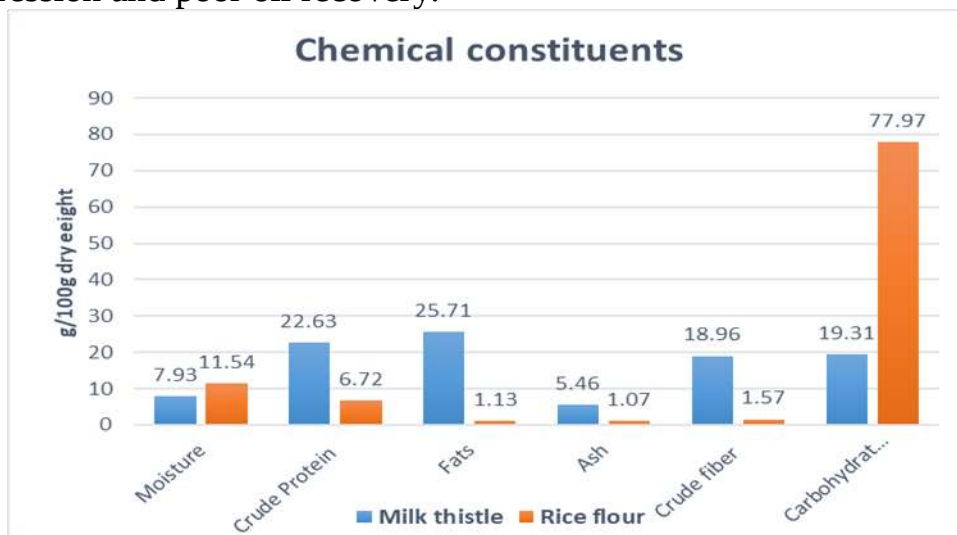


Fig. (1) Chemical composition of milk thistle and rice flour

Table (1): showed the nutritional impact of supplementing rice flour with increasing concentrations (5-25%) of milk thistle (*Silybum marianum*). There are significant differences ($p < 0.05$) in groups. Protein content increased from 6.73% in the control to 7.91% at 25% substitution, which may be attributed to the higher protein content in milk thistle seeds. According to **El-haak et al. (2015)**, *Silybum marianum* seeds contain about 20–25% protein, making them a suitable fortifying agent for cereal-based products.

Similarly, ash and fiber contents increased with milk thistle addition, consistent with the mineral and dietary fiber-rich composition of milk thistle as documented by **Apostol et al. (2017)**, who reported high levels of calcium, magnesium, and insoluble fibers. The fat content also increased significantly, in line with previous findings that milk thistle seeds are rich in lipids, particularly unsaturated fatty acids (**Dogan et al., 2022**). Conversely, carbohydrate content decreased, which is expected due to the displacement of rice flour, which is predominantly starchy. Although the reduction in carbohydrate slightly lowered the energy values, the decrease was minimal and not nutritionally concerning. Energy values remained within the range of 414–417 kcal/100g,

aligning with the typical caloric contribution of cereal and seed blends (Meddeb et al., 2017). Moisture content slightly increased with milk thistle substitution. This could be explained by the more hygroscopic nature of fiber-rich flours and the presence of bioactive compounds in milk thistle that may retain water (Mirhosseini, and Amid 2012). The enhancement in nutritional parameters without a drastic decline in energy value suggests that milk thistle flour can be a valuable additive in the development of functional food products.

Table (1): proximate chemical composition of biscuit (g/100g dry weight)

Treatment	Protein	Ash	Fiber	Fat	Carbohydrates	Moisture	EnergyCal./100g
Cont.	6.73±0.14 ^f	1.26±0.03 ^a	2.32±0.06 ^a	9.55±0.07 ^a	76.00±0.10 ^a	4.14±0.04 ^a	416.89±1.59 ^a
T1	7.32±0.07 ^d	1.46±0.04 ^b	2.51±0.05 ^b	9.80±0.08 ^b	74.65±0.25 ^b	4.26±0.04 ^b	416.28±1.98 ^a
T2	7.52±0.02 ^c	1.55±0.04 ^c	2.68±0.05 ^c	9.93±0.04 ^b	73.93±0.18 ^c	4.39±0.07 ^c	415.15±1.16 ^b
T3	7.73±0.03 ^b	1.61±0.03 ^d	2.75±0.03 ^d	10.12±0.07 ^c	73.28±0.17 ^c	4.51±0.04 ^d	414.27±1.44 ^b
T4	7.86±0.03 ^a	1.73±0.04 ^e	2.83±0.04 ^e	10.23±0.07 ^d	72.75±0.13 ^d	4.60±0.03 ^d	414.40±1.23 ^b
T5	7.91±0.07 ^a	1.79±0.04 ^e	2.90±0.03 ^f	10.42±0.06 ^e	72.27±0.16 ^d	4.71±0.02 ^e	414.50±1.41 ^b

Values are expressed as mean ± SD, the same letters within the same column are not significant ($P \leq 0.05$) for each characteristic. Cont.: 100% RF, T1: (95% RF+5% MTF), T2: (90% RF+10% MTF), T3: (85% RF+15% MTF), T4: (80% RF+20% MTF) and T5: (75% RF+25% MTF)

Table (2) showed significantly differences between milk thistle seeds and rice flour in minerals content. Milk thistle showed significantly higher calcium levels (187.38 mg/100g). compared to rice flour (22.54 mg/100g). Calcium content in milk thistle is about 8 times thus supports the findings of Karkanis et al. (2018) who reported that calcium concentrations of 150-210 mg/100g in Silybum marianum seeds. The low calcium in rice flour is typical of refined cereals, as noted by Kennedy and Burlingame (2003) in their FAO nutritional analysis of rice products.

The exceptionally high potassium content in milk thistle (690,05 mg/100g vs 120.47 mg/100g in rice flour) corroborates with data from (Alkhalf and Al-Khalifa 2017). This reflects milk thistle's adaptation to arid environments where potassium accumulation aids osmotic regulation, while polished rice loses potassium-rich aleurone layers during milling (Juliano, 2007).

Magnesium is about 3 times higher in milk thistle than rice flour (132.13 vs 41.57 mg/100g) and phosphorus is 6 time higher in milk thistle than in rice flour (675 vs 113.93 mg/100g). These minerals are crucial for seed germination and secondary metabolite production. Rice flour's lower values reflect the removal of nutrient-dense bran layers (Saunders, 1990).

The remarkable iron content in milk thistle (11.3 mg/100g) versus rice flour (1.57 mg/100g) is particularly noteworthy. This aligns with García-Moreno et al. (2013) who found 8-12 mg/100g iron in Spanish milk thistle populations. While rice is typically low in iron (Kobayashi et al. 2014).

The mineral density of milk thistle supports its usage as an excellent nutritive source. As Wallace et al. (2003) demonstrated, these minerals likely contribute to its hepatoprotective effects by supporting enzymatic pathways. The mineral depletion in rice flour highlights the nutritional consequences of refining processes, well-documented by Kennedy and Burlingame (2003).

Table (2): Minerals content in raw material (Milk thistle and Rice flour)

Minerals	Milk thistle mg/100g dry weight	Rice flour mg/100g dry weight
Calcium (Ca)	187.38	22.54
Potassium (K)	690.05	120.47
Magnesium (Mg)	132.13	41.57
Phosphorus (P)	٦٧٥	113.93
Iron (Fe)	11.3	1.57

Results in **Table (3)** and **Fig. (2)** showed the comparative analysis of bioactive compounds and antioxidant activities between milk thistle (*Silybum marianum*) and rice flour reveals significant differences that align with their distinct phytochemical profiles and biological functions. Milk thistle has significantly more total phenolics (6.64 ± 0.08 mg GAE/g) and flavonoids (3.87 ± 0.11 mg RE/g) than rice flour (1.15 ± 0.04 mg GAE/g and 0.80 ± 0.05 mg RE/g, respectively). These results are compatible with: **Karkanis et al. (2018)** found 5.2-7.8 mg GAE/g phenolics in milk thistle seeds, attributed to silymarin complexes. **Ti et al. (2014)** discovered 0.9-1.3 mg GAE/g in refined rice flour due to bran removal during processing.

Other results from the same table are antioxidant activity using DPPH. The DPPH radical scavenging assay showed concentration-dependent effects:

At 50-100 $\mu\text{g/mL}$, milk thistle showed superior activity (48.54-53.57%) versus rice flour (45.78-48.70%), correlating with its higher phenolic content

The crossover at 150 $\mu\text{g/mL}$ (milk thistle: 59.58% vs rice flour: 64.61%) suggests Possible pro-oxidant effects of milk thistle compounds at high concentrations, as observed by **Foti et al. (2019)** for some flavonoids (Non-phenolic antioxidants (e.g., γ -oryzanol) in rice flour becoming dominant at higher doses (**Patel and Naik, 2004**). The IC₅₀ values demonstrate clear efficacy differences Milk thistle (158.8 ± 0.12 $\mu\text{g/mL}$) showed intermediate activity, comparable to reports by **Abenavoli et al. (2018)** for silymarin-rich extracts. Rice flour (235.53 ± 0.16 $\mu\text{g/mL}$) exhibited weaker activity, typical for refined cereals (**Zhang et al., 2015**) BHT (58.17 ± 0.14 $\mu\text{g/mL}$) served as an effective synthetic reference, matching literature values (**Huang et al., 2005**) The unexpectedly high IC₅₀ for vitamin C (235.5 ± 0.11 $\mu\text{g/mL}$) contradicts established literature where L-ascorbic acid typically shows IC₅₀ <50 $\mu\text{g/mL}$ (**Carr and Frei, 1999**). Degradation during sample preparation (pH >4 accelerates oxidation, as shown by **Nyyssonen et al., 2000**. The superior antioxidant profile of milk thistle supports its traditional use in hepatoprotection, aligning with findings by **Vargas-Mendoza et al. (2014)** and **Surai (2015)**

While most results are scientifically valid, the vitamin C data requires methodological review.

Table (3): Antioxidant activity (DPPH inhibition) of Milk thistle and Rice flour

Antioxidant activity (DPPH inhibition)	Milk thistle	Rice flour	V.C	BHT
50	48.54 $\pm$ 0.15	45.78 $\pm$ 0.25	45.78 $\pm$ 0.32	50.06 $\pm$ 0.34
100	53.57 $\pm$ 0.23	48.70 $\pm$ 0.43	48.70 $\pm$ 0.47	64.61 $\pm$ 0.51
150	59.58 $\pm$ 0.46	64.61 $\pm$ 0.18	64.61 $\pm$ 0.18	68.18 $\pm$ 0.29
IC ₅₀	158.8 $\pm$ 0.12	235.53 $\pm$ 0.16	235.5 $\pm$ 0.11	58.17 $\pm$ 0.14

Values are expressed as mean $\pm$ SD, the same letters within the same column are not significant ($P \leq 0.05$) for each characteristic

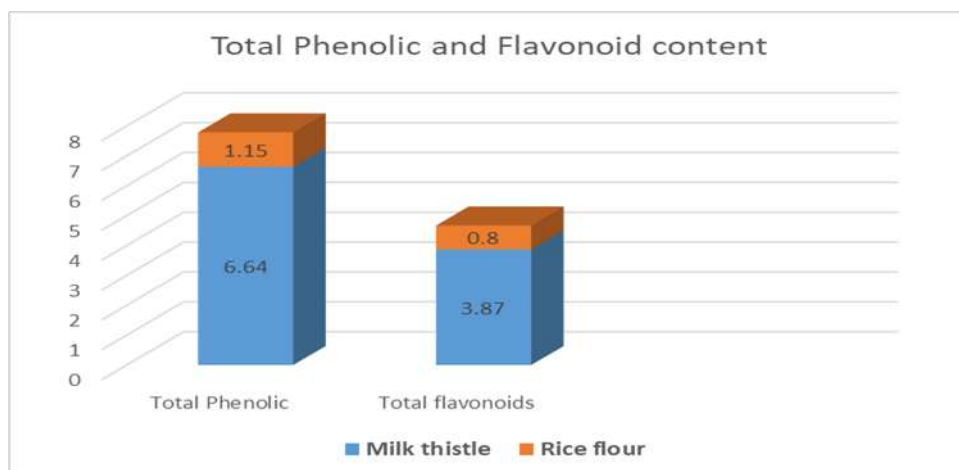


Fig. (2): Total phenolic (mg GAE/g) and Total flavonoids (mg RE/g) in Milk thistle and Rice flour

The color parameters (L^* , a^* , b^*) and total color difference (ΔE^*) of gluten-free biscuits were significantly influenced by the substitution of rice flour with milk thistle flour. The control sample (100% rice flour) exhibited the highest L^* value (78.82 ± 0.2), indicating a lighter appearance. As the proportion of milk thistle flour increased from T1 to T5, a marked decrease in L^* values was observed, reaching 54.4 ± 0.26 in T5, indicating a darker color. This trend is consistent with findings by **Pathare et al. (2013)**, who noted that L^* values decrease with increasing levels of colored or pigmented ingredients.

The progressive increase in both a^* (redness) and b^* (yellowness) values across treatments indicates a shift towards a more reddish-brown hue. This color development may be attributed to the Maillard reaction and caramelization processes during baking, which are enhanced by the presence of proteins and phenolic compounds in milk thistle flour. According to **Błaszczak et al. (2021)**, higher levels of protein and polyphenols can intensify browning reactions during thermal processing.

Furthermore, ΔE^* values increased significantly with each level of substitution, indicating visible and measurable color differences compared to the control. ΔE^* values greater than 3.0 are generally perceptible to the human eye, while values exceeding 5.0 are clearly noticeable (**Pathare et al., 2013**). The highest ΔE^* value (25.38 ± 0.3 in T5) confirms a substantial change in visual appearance, which could influence consumer perception.

These findings are in agreement with **Radović et al. (2022)**, who reported that increasing incorporation of milk thistle flour in baked products alters their surface color due to its natural pigmentation, mainly derived from flavonolignans such as silybin and silychristin. The darker color may also reflect the antioxidant-rich profile of milk thistle, which is beneficial from a nutritional standpoint but may require sensory adaptation.

Table (4): Color characteristics of gluten-free rice biscuits replaced with milk thistle seeds

Treatment	L^*	a^*	b^*	ΔE^*
Control	$78.82 \pm 0.2a$	$1.36 \pm 0.14d$	$1.36 \pm 0.14d$	$0.01 \pm 0.005f$
T1	$71.28 \pm 0.25b$	$0.35 \pm 0.11e$	$0.35 \pm 0.11e$	$8.24 \pm 0.18e$
T2	$66.46 \pm 0.41c$	$1.37 \pm 0.22d$	$1.37 \pm 0.22d$	$12.45 \pm 0.4d$
T3	$61.97 \pm 0.6d$	$2.23 \pm 0.2c$	$2.23 \pm 0.2c$	$17.74 \pm 0.3c$
T4	$58.85 \pm 0.7e$	$3.42 \pm 0.29b$	$3.42 \pm 0.29b$	$21.52 \pm 0.4b$

T5	54.4±0.26f	3.77±0.3a	3.77±0.3a	25.38±0.3a
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Data are presented as means $\pm$ SD (n=3). a, b, c, d, e and f: Means with different letters among treatments in the same column are significantly different (P < 0.05) Cont.: 100% RF, T1: (95% RF+5% MTF), T2: (90% RF+10% MTF), T3: (85% RF+15% MTF), T4: (80% RF+20% MTF) and T5: (75% RF+25% MTF)

Table (5) indicated the incorporation of milk thistle flour resulted in a progressive reduction in both fracturability and adhesiveness, with statistically significant differences across treatments ($p < 0.05$). The control sample (100% RF) exhibited the highest fracturability and adhesiveness values, while the 25% MTF sample recorded the lowest. This is consistent with the fact that rice flour, being starch-rich, contributes to a cohesive and elastic structure, while milk thistle flour, rich in fiber and bioactives, disrupts matrix formation (Hager et al., 2012; Ktenioudaki et al., 2012).

The hardness cycle also showed a distinctive pattern: values remained low for lower substitution levels (5–10% MTF), but increased sharply at higher levels (15–25%). This may indicate a change in the textural nature of the product, becoming harder and more rigid due to excessive fiber content and less gelatinized starch interaction (Sabanis and Tzia, 2011). Dietary fibers absorb water and create a dense structure, which reduces cell expansion and increases resistance during the first compression cycle (Rosell et al., 2001).

The reduction in adhesiveness is also noteworthy, as it suggests a drier and less cohesive product with increasing MTF. This could be due to the low starch content and high insoluble fiber in milk thistle, which limits the formation of a sticky or elastic crumb structure (Gallagher et al., 2004).

These results align with previous findings where incorporation of high-fiber, low-gluten or non-gluten plant materials altered texture parameters negatively, unless counterbalanced by hydrocolloids or binding agents (Mildner-Szkudlarz et al., 2013).

Table (5): Texture profile of gluten-free rice biscuits replaced with milk thistle seeds

Hardness Cycle	Fractur ability	Adhesiveness	Treatment
0.05±0.04c	33.55±1.2a	33.59±1.18a	Cont.
0.046±0.04c	25.26±0.5b	25.26±0.5b	T1
0.02±0.01c	24.12±0.63c	24.08±0.6c	T2
0.17±0.14b	23.00±0.29d	23.17±0.41d	T3
0.2±0.1a	20.97±0.36e	21.25±0.6e	T4
0.2±0.1a	9.82±0.5df	19.29±0.2f	T5

Data are presented as means $\pm$ SD (n=3). a, b, c, d, e and f: Means with different letters among treatments in the same column are significantly different (P < 0.05) Cont.: 100% RF, T1: (95% RF+5% MTF), T2: (90% RF+10% MTF), T3: (85% RF+15% MTF), T4: (80% RF+20% MTF) and T5: (75% RF+25% MTF)

Results in Table (6): showed the incorporation of milk thistle (*Silybum marianum*) seed flour into rice flour-based biscuits significantly influenced the sensory attributes, including color, texture perception, and overall acceptability. The control sample made with 100% rice flour exhibited the highest scores for all sensory parameters, including color (18.1), crumb color (18.05), surface character (17.00), mouthfeel (16.21), and overall acceptability (17.00). These findings align with previous reports that rice flour provides a light color and smooth texture, which are generally well-accepted by consumers (Ju et al., 2020).

As the substitution level of milk thistle flour increased, a progressive decline in color scores was observed, dropping to 9.44 at the 25% replacement level. This darkening effect is likely attributed to the high content of polyphenolic compounds and

pigments naturally present in milk thistle seeds (ROSÍŃSKA et al., 2017), which affect the visual appeal of baked goods. Similarly, crumb color and surface character values also decreased significantly with increasing milk thistle content, indicating a change in the internal structure and surface uniformity of the biscuits.

Break shred values, conversely, increased with higher milk thistle levels (from 10.78 in the control to 16.00 at 25%), suggesting increased brittleness. This is likely due to the high fiber content and lower starch concentration of milk thistle flour, which may reduce dough cohesiveness and water retention during baking, leading to a crumblier texture (Ahmed et al., 2021).

Mouthfeel and overall acceptability scores followed the same declining trend, with the lowest values recorded at 25% substitution (10.45 and 9.66, respectively). These declines are consistent with studies that show high fiber or protein-rich flours, when added in large amounts, can negatively affect sensory qualities such as texture, taste, and aftertaste due to coarse particles and bitter compounds (Rosell et al., 2009 and Hager et al., 2012).

The data suggest that lower levels of substitution, particularly between 5% and 10%, may enhance nutritional value without compromising sensory acceptability. At these levels, the biscuits maintained relatively high scores across most sensory parameters while benefiting from the antioxidant and bioactive properties of milk thistle, including silymarin content known for its health-promoting effects (Karkanis et al., 2011).

Table (6): Sensory properties scores of rice biscuits samples

Overall Acceptability	Mouth Feel	Surface Character	Crumb Color	Break Shred	Color	Treatments
17.00±0.2a	16.21±0.01a	17.00±0.21a	18.05±0.2a	10.78±0.1f	18.1±0.2a	Cont
15.12±0.2b	15.22±0.06b	16.33±0.11b	16.00±0.02b	12.01±0.2e	16.05±0.02b	T1
14.64±0.04c	14.00±0.3c	15.00±0.14c	14.33±0.2c	13.25±0.1d	14.00±0.1c	T2
12.22±0.07d	13.47±0.01d	14.66±0.04d	.65±0.22d ³ 1	14.00±0.1c	12.55±0.4d	T3
10.00±0.4e	11.00±0.5e	12.44±0.03e	10.00±0.01e	15.22±0.1.6b	10.64±0.2e	T4
.66±0.1f ³	10.45±0.03f	10.00±0.07f	.95±0.4f ³	16.00±0.03a	.44±0.5f ³	T5

Data are presented as means ± SD (n=20). a, b, c, d, e and f: Means with different letters among treatments in the same column are significantly different (P < 0.05) Cont.: 100% RF, T1: (95% RF+5% MTF), T2: (90%RF+10% MTF), T3: (85% RF+15% MTF), T4: (80% RF+20% MTF) and T5: (75% RF+25% MTF)

Conclusion

The substitution of rice flour in rice-based biscuits with milk thistle flour improved their nutritional profile, particularly in terms of protein, dietary fiber, and essential minerals (calcium, potassium, magnesium, phosphorus, and iron). However, increasing substitution levels (5–25%) led to notable changes in physicochemical and sensory properties, including darker coloration, increased hardness, reduced adhesiveness, and lower consumer acceptability at higher concentrations. The best inclusion amount appears to be 5-10% milk thistle flour, since this range increases nutritious content without significantly reducing texture, color, or sensory appeal. Higher replacement levels (15-25%) produced a denser, more brittle product which may necessitate further formulation changes (such as hydrocolloids or flavor masking) to increase consumer approval. These findings indicated that milk thistle's potential as a functional food

component for gluten-free goods, especially for those looking to increase their protein, fiber, and antioxidant intake.

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