

## **Production of high nutrients and antioxidants gluten and lactose-free cupcake using beetroot powder and coconut milk.**

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### **ABSTRACT:**

Antioxidant compounds play a vital role in celiac disease patients, previous studies have proven the correlation between celiac disease and increased incidence oxidative stress in people with celiac, red beetroot and coconut as traditional, high-antioxidant and anti-inflammatory capacity of certain popular compounds suggest their potential therapeutic value in the treatment of numerous diseases.

This study aims to point out the impact of the substitution of gluten-free flour with various levels (0, 15, 30 and 45%) of beetroot powder as source of dietary fiber, minerals and phytochemical nutrients, also substitution of cow milk by coconut milk on nutritional indicators and sensorial evaluations in gluten and lactose-free cupcake. Data revealed that all substituted cupcake sample showed significant increase at  $P < 0.01$  in ash, fiber and moisture content. While found significant decrease of total carbohydrates and fat in cupcake fortified by 30 and 45% compared with the control. Also found high significant increase of Mg, Ca, Fe, K, Zn and Na at  $P < 0.01$  in cupcake sample fortified by 30 and 45% beetroot powder compared with control sample.

The results found high significant increase at  $P < 0.01$  in total phenolic and antioxidant activity level in all cupcake sample fortified by beetroot powder compared with control sample.

The sensorial evaluation data showed that blends of coconut milk and beetroot powder at level 15 and 30% were improved crumb color, flavor and taste and overall acceptability.

## Introduction:

Celiac disease, a gluten-sensitive inflammatory condition affecting approximately 1% of the worldwide population, is characterized by a chronic inflammatory response to gluten ingestion in susceptible individuals, as mentioned by *Arentz-Hansen et al. (2004)*. Gluten is a protein actually contents of two main groups of proteins: gliadins and glutenins (*Book et al., 2003*). Gluten, which is found in wheat, rye, and barley, presents a significant challenge for individuals with celiac disease due to its ubiquitous, tasteless, and often invisible nature. Many patients prepare gluten-free meals in kitchens where gluten-containing foods are also present, leading to a common fear of cross-contamination. This fear can result in hypervigilance and reduced quality of life (that's by *Wolf et al., 2018*).

In multiple studies, a positive correlation between celiac disease and the generation of reactive oxygen species (ROS) in the body, resulting in oxidative stress, has been demonstrated (*Kashyap et al., 2022*).

In patients with celiac disease, the excessive production of (ROS) occurs as a result of gluten ingestion. This initiates a series of reactions leading to oxidative stress, which affects not only the small intestinal wall but also impacts the entire body. The oxidative stress resulting from this process is responsible for damaging essential cellular components, ultimately altering their functions negatively (*Boda et al., 1999*).

*Odetti et al. (1998)* mentioned that an imbalance between ROS production and antioxidant capacity is observed in individuals with untreated celiac disease. The reduced antioxidant potential may reflect an increased demand to counteract the elevated ROS levels.

*Rowick et al. (2018)* reported that oxidative stress is implicated in the development of celiac disease CD and may play a role in both the disease's course and the development of related complications.

The body is protected from free radical damage by a network of efficient antioxidant defenses, including both enzymatic and non-enzymatic mechanisms. Total antioxidant capacity (TAC) provides a comprehensive assessment of the body's ability to neutralize these damaging free oxygen radicals as reported by (*Spiteller, 2007; Stojiljković et al. 2009 & Nahla et al. 2018*).

Oxidation is a major cause of food quality deterioration, affecting nutritional value, texture, and appearance, and leading to off-flavors and potentially toxic compounds in food (*Dong et al., 2008*). Antioxidants are therefore crucial in both food preservation and human health to counteract oxidative stress (*Li et al., 2018*).

The global consumption of bakery products is on the rise, with cake being one of the most popular and enjoyed options among people of all ages (*Douati et al., 2017*). The high demand for these products can be attributed to their sensory appeal, practicality, and convenience, as consumers increasingly prefer easy-to-prepare meals that meet their taste expectations (*Manisha et al., 2012*).

Red beetroot, commonly referred to as beet, garden beet, or table beet, is a vegetable with a long history of consumption in diverse culinary traditions worldwide. Its taxonomic classification places it within the *Beta vulgaris* subspecies, specifically the *Beta vulgaris* ssp. *vulgaris* L. This subspecies is a member of the *Beta* L. genus, which, in turn, belongs to the *Betoideae* subfamily. The *Betoideae* subfamily is part of the *Amaranthaceae* family, also known as the goosefoot family. Finally, red beetroot is classified within the *Caryophyllales* order (*Ceclu and Nistor, 2020*). *Neelwarne and Halagur (2013)* mentioned that plants of the *Beta* genus are thought to have originated in North Africa and subsequently dispersed via the Mediterranean Sea route, eventually establishing themselves along the coastlines of Europe, Asia, and the Americas.

*Ceclu and Nistor (2020)* reported that beetroot is a globally consumed vegetable recognized for its rich content of bioactive compounds, including betalains, inorganic nitrates, polyphenols, folates, and various essential vitamins and minerals found in its tuberous root. Similar to turnips, both the leaves and roots of the beet are edible. Depending on the region, beetroot can be enjoyed in various forms, such as whole, cooked, canned, or minimally processed. It is utilized not only as a vegetable but also for its juice and extracts, which are employed in traditional medicine, as food colorants, and in cosmetic products. This plant is noted for its strong antioxidant and anti-inflammatory properties, suggesting its potential role in aiding the treatment of several health conditions.

Also, beetroot is known for its beneficial medical properties, including anti-inflammatory, anti-allergenic, antiplatelet, antiatherogenic, cardioprotective, and vasodilatory effects (*Balasundram et al., 2006*). One of the key quality attributes that attracts consumers to edible products is color, which serves as a crucial sensory characteristic for consumer acceptance. Due to health risks associated with synthetic dyes-particularly their potential role in the onset and progression of chronic diseases affecting liver and kidney function-there is a growing caution regarding their use in food manufacturing (*Sahar and Manal, 2012*).

*Theba et al. (2020)* reported that beetroot is widely utilized in the food industry as a natural additive due to its non-toxic pigments and coloring properties. Beetroot powder offers various functional characteristics, including water holding capacity, water retention, swelling capacity, and oil absorption, making it suitable for incorporation into a range of food products such as cookies, cakes, snacks, and candies. Additionally, beetroot pomace is a cost-effective option that can serve as a valuable functional ingredient.

The coconut palm (*Cocos nucifera* L.) is a valuable economic plant primarily grown in tropical regions, particularly in Asia. It is abundant in fiber, vitamins, and minerals and is naturally gluten-free. Additionally, it aids in digestion and enhances the absorption of essential nutrients. Coconut flour, a key by-product of coconut milk, plays an important role, as reported by (*Ramya and Anitha, 2020*) & (*Ramaswamy, 2014*).

Coconut milk is typically obtained by pressing or squeezing grated coconut meat, with added water or without. It is a key component in various culinary dishes, including curries and desserts (*Tansakul and Chaisawang, 2006*), and serves multiple purposes beyond just being a food ingredient.

Coconut milk is highly nutritious rich in vitamins and minerals as mentioned by (*Vudugula and waghray, 2018*). In a study of *Alyaqoubi et al. (2015)* examining antioxidant activity and physicochemical properties, coconut milk samples showed statistically significant ( $P < 0.05$ ) differences in antioxidant activity compared to goat and cow milk, as reported by *Alyaqoubi et al. (2015)*.

Thus, the goal of this research was to investigate the impact of substitution of gluten free flour by different levels (0, 15, 30 and 45%) of beetroot powder as source of dietary fiber, minerals and phytochemical nutrients, also substitution of cow milk by coconut milk as source of minerals and phytochemical nutrients on nutritional indicators and sensorial evaluations in gluten and lactose-free cupcake.

## Material and methods:

### Materials:

Dry beetroot was obtained from minnie's market, commercial gluten free rice flour, gluten free corn flour, crushed coconut, corn oil, vanillin, egg, baking powder, salt and Arp gum were acquired from the local market of Sohag City, Egypt.

### Preparation of coconut milk:

Coconut milk was prepared according method of *Mepba et al., (2009)*. Meanwhile, a 1:3 (w/v) mixture of ground coconut meat and water was prepared, and the resulting slurry was filtered through double cheesecloth layers.

### Preparation of beetroot flour:

The dried beetroot was processed using a household mincer (Moulinex, Super Blender, France) to create beetroot flour. This flour was then stored in polyethylene bags until it was needed for cupcake preparation.

### Preparation and formulation of gluten free cupcake sample:

Gluten free cupcake samples were prepared according to *A.A.C.C (2000)* using the formula as described by *Abd Rabou and Al-Sadek, (2018)* with some modifications. Table (1) summarized all cupcake sample formulations, gluten free corn flour and rice flour was substituted by 15, 30 and 45% of beetroot powder. Meanwhile all row ingredients of each cupcake sample was mixed gently until got homogenous dough using electric egg mixer (Supermix 150, *Moulinex Compact*, France). Once the dough reached the desired texture, it was placed in paper cups to bake at  $180^{\circ}\text{C} \pm 5^{\circ}\text{C}$  for 12 to 20 minutes. After baking, the cupcakes were allowed to cool at room temperature inside a sealed plastic bag and then stored in the refrigerator for the time to analyze at  $5^{\circ}\text{C}$ .

Table (1). Formulation of prepared cupcake sample:

Ingredients	Cupcake sample			
	Control sample (0%)	beetroot powder (15%)	beetroot powder (30%)	beetroot powder (45%)
Corn flour gm	75	63.75	52.5	41.25
Rice flour gm	75	63.75	52.5	41.25
Beetroot powder gm	-	22.5	45	67.5
Salt gm	1	1	1	1
Sugar gm	80	80	80	80
Arab gum gm	2	2	2	2
Baking powder gm	5	5	5	5
Corn oil ml	50	50	50	50
Egg gm	60	60	60	60
Coconut milk ml	65	65	65	65
Vanil gm	0.5	0.5	0.5	0.5

**Nutritional analysis:**

The moisture, fat, crude protein, crude fiber, and ash contents for all samples were analyzed using established methods. Total carbohydrates were calculated by difference.

**Determination of minerals:**

The elements Ca, Fe, Zn, and Mg were analyzed using an Inductively Coupled Plasma Emission Spectrometer (iCAP 6200) (*Isaac and Johnson, 2002*). Sodium and potassium levels were measured with a Flame Photometer (Jenway PFP7) following standard procedures pointed out by *AOAC (2005)*.

**Determination of total phenolics and antioxidant activity:**

The Folin-Ciocalteu method, as described by Singleton and Rossi (1965), was used to determine total phenolic content. The DPPH assay, based on the method of *Brand-Williams et al. (1995)*, was performed with specific modifications.

**Sensorial evaluation of prepared gluten free cupcake:**

Sensory evaluation of cupcake samples, including crust and crumb color, crumb hardness, taste, flavor, and overall acceptability, was conducted after cooling them for 1 to 2 (h), at room temperature (25°C) in sealed plastic bags.

**Statistical analysis:**

The analysis of data was utilizing the Statistics Package for Social Sci. (SPSS, 20), data were reported as mean  $\pm$  standard error of means ( $n = 3$ ) for all chemical analysis and ( $n=7$ ) for sensorial evaluation. Differences between means were determined by analysis of variance (ANOVA) with LSD post hoc test,. Declared the significance at  $P < 0.05$  and  $P < 0.01$  (*Pallant, 2005*).

**Results and discussion:****Nutritional indicators of prepared gluten free cupcake:**

Table 2 points out the chemical composition of cupcake substitution with variations in level of beet roots powder. Data revealed that all substitution cupcake sample showed significant increase at  $P < 0.01$  in ash, fiber and moisture content. While found significant decrease of fat, total carbohydrates and caloric value in cupcake substituted by 30 and 45% compared with the control.

Meanwhile, results recorded significant increase at  $P < 0.05$  in nitrogen value but non - significant in protein f value found in cupcake substituted by 45% beet roots powder.

This results agree with *Li et al. (2018)* how reported that coconut cake represents a promising and abundant source of edible protein, with potential applications for its protein fractions in functional food development.

*Dhawan and Sharma (2019)* how revealed that beetroot flour is a valuable source of carbohydrates, crude fiber, iron, calcium, and vitamin C. Therefore, it could serve as a beneficial ingredient in various dishes to improve the nutritional content of food.

*Alshehry (2019)* evaluated the chemical analysis of beetroot powder, including assessments of total phenolics, flavonoid compounds, antioxidant activity, mineral content, and the identification of betalain pigments using HPLC. Cupcakes were prepared with 2.5%, 5.0%, 7.5%, and 10% beetroot powder substituted for 72% extraction wheat flour. The findings indicated that beetroot powder is a rich source of crude fiber (20.4%), total phenolics, flavonoids, and antioxidant activity. Additionally, potassium and sodium were the predominant minerals, and betalains were the major pigments identified from beetroot powder.

**Table (2):** Chemical composition of gluten free cupcake substitution with 0, 15, 30, 45% of beetroot powder % on wet weight basis

Component Cupcake Sample	Nitrogen	Moisture	Ash	Protein	Fat	Fiber	Total carbohydrates*	Caloric value/100 g
Control (0%)	1.23±0.09	23.20±0.06	2.05±0.03	8.14±0.26	16.9±0.05	0.61±0.005	48.4±0.74	378.24±2.96
Beetroot (15%)	1.31±0.06	25.0*±.05	2.05±0.05	8.24±0.44	16.4±0.37	0.62±0.005	46.40±1.5	365.96*±3.98
Beetroot (30%)	1.34±0.03	26.75*±0.66	2.75*±0.07	8.42±0.52	16.27*±0.003	0.67*±0.03	45.12±0.84	360.68**±2.31
Beetroot (45%)	1.41*±0.03	27.56*±0.98	3.0**±0.05	8.40±0.06	16.27*±0.10	0.97**±0.01	43.65**±1.13	354.70**±5.22

\*Carbohydrates were calculated by difference.

Values are presented as means ± standard error of mean.

Significant \*P < 0.05, highly significant \*\* P ≤ 0.01.

**Minerals content of different prepared gluten free cupcake:**

Minerals content of gluten free cupcake are shown in table (3), meanwhile results recorded high significant increase of Mg, Ca, Fe, K, Zn and Na at  $P < 0.01$  in cupcake sample substituted by 30 and 45% beetroots powder compared with control group.

This results agree with the results obtained by *Ranawana et al. (2018)* how found significant increase of protein, fibre, potassium, magnesium, phosphorus, iron, folate and water content in sponge cake beetroots compared to control sample.

*Manopriya and Arivuchudar (2019)* how found significant increase of carbohydrate, protein, energy, crude fiber, iron, calcium and sodium in all cake sample fortified by beetroot powder compared with standard sample.

**Table (3):** Minerals content of gluten free cupcake substitution with 0, 15, 30, 45% of beetroot powder

Minerals Cupcake Sample	Mg Ppm	Ca Ppm	Fe Ppm	Zn Ppm	Na %	K %
Control sample (0%)	354.4± 4.2	235.0± 2.88	209.33± 2.49	7.90± 0.60	0.74± 0.003	0.41± 0.006
Beetroot (15%)	354.53± 3.54	370.0** ± 5.78	219.24**± 0.44	11.11** ± 0.60	0.76± 0.14	0.48± 0.02
Beetroot (30%)	389.17** ± 6.26	420.0** ± 0.57	442.58**± 1.48	11.34** ± 0.38	0.79** ± 0.008	0.56**± 0.006
Beetroot (45%)	492.40** ± 4.39	460.0** ± 0.58	244.67**± 0.2	12.25** ± 0.14	0.85** ± 0.006	0.62**± 0.05

Values are presented as means ± standard error of mean.

Significant \* $P < 0.05$ , highly significant \*\* $P \leq 0.01$ .

**Total phenols and antioxidant activity of gluten free cupcake:**

Table (4) showed that total phenolic and antioxidant activity of gluten free cupcake. The results found high significant increase at  $P < 0.01$  in total phenolic and antioxidant activity level in all cupcake sample substituted by beetroot powder compared with control sample. This results according to the results of *Kujala et al. (2000)* reported that beetroot is a rich source of various phenolic acids, including ferulic, protocatechuic, vanillic, p-coumaric, p-hydroxybenzoic, and syringic acids. Additionally, *Yao et al. (2004)* reported that beetroot is known to contain high levels of phenolic compounds, which function as antioxidants and may contribute to the prevention of tumors and cardiovascular diseases.

*Seneviranta and Dissanayake (2005)* determined the phenolic antioxidant in coconut cake, found total phenolic content in coconut cake



was  $2156 \pm 16$  mg/Kg. Also with (*Mahfouz and Abd-Elnoor, 2017*) it points out that beetroot are rich in phenolic compounds, flavonoids and antioxidant activity.

*Ranawana et al. (2018)* found that beetroot supplementation significantly enhances the antioxidant and polyphenol content of sponge cake, improving oxidative stability and potentially extending shelf life. These benefits may be further amplified by combining beetroot with other natural products.

Also agree with *Theba, et al. (2020)* who mentioned that beetroot offers a wealth of betalains and phytochemicals, making it a versatile ingredient for food products. Furthermore, the abundant pomace generated during processing is a cost-effective source of dietary fiber, bioactive compounds, and antioxidant properties.

*Mistriuanu et al. (2022)* explored the use of antioxidant-rich beetroot peel powder (BPP) at concentrations of 1.5%, 3%, 5%, and 7% to develop a value-added mayonnaise. The BPP exhibited high betalain ( $1.18 \pm 0.03$  mg/g DW) and polyphenolic ( $225.36 \pm 1.97$  mg GAE/g DW) content, along with significant antioxidant activity.

**Table (4):** Total phenolic and antioxidant activity of gluten free cupcake substitution with 0, 15, 30, 45% of beetroot powder

Parameter Cupcake sample	Total phenolic compounds (mg GAE/ g extract)	Antioxidant Activity %
Control sample (0%)	$8.69 \pm 0.35$	$2323.5 \pm 71.88$
Beetroot (15%)	$10.07^{**} \pm 0.23$	$3255.0^{**} \pm 55.43$
Beetroot (30%)	$11.46^{**} \pm 0.29$	$3375.0^{**} \pm 69.28$
Beetroot (45%)	$25.10^{**} \pm 0.05$	$16395.0^{**} \pm 25.98$

Values are presented as means  $\pm$  standard error of mean.

Significant \* $P < 0.05$ , highly significant \*\* $P \leq 0.01$ .

### Sensory characteristics of cupcake produced from gluten free flour substitution with beetroot powder:

Sensory characteristics of cupcake at different levels of gluten free flour substituted with beetroot powder that point in Table (5). The outcomes found no substantial variations among all samples at  $p < 0.05$ , in all sensory characteristics indicated of cupcake. At the same time results showed cupcake produced with 30% of beetroot powder was rated like moderately in terms of taste and flavor and overall acceptability which compared favorably with the control cupcake. The second best sample was the cupcake produced with 15% of beet roots powder which also

compared favorably with the control cupcake, while the worst sample in terms of crust color, crumb color, crumb hardness, and taste and flavor and overall acceptability was the cupcake produced with 45% beetroot powder.

This results according to the result of *Paucean et al. (2016)* how gluten-free cookies with desirable flavor and taste can be achieved through rice and coconut flour blends. Moreover, coconut flour's inherent nutritional qualities make it a valuable addition for enhancing the nutritional profile of goods baked.

Cookies formulated with a 10% substitution of beetroot powder for wheat flour exhibited enhanced color and appearance, according to *Ingle et al. (2017)*. While increasing the beetroot powder content darkened the ground color, a 10% replacement yielded optimal acceptability.

Also agree with *Alshehry (2019)* how found substituting up to 10% of the cupcake batter with beetroot powder resulted in optimal sensory characteristics, including color for both the crust and crumb. This substitution also effectively inhibited the growth of bacteria, fungi, and molds in the cupcakes at this same 10% level.

**Table (5):** Sensory characteristics of gluten free cupcake substitution with 0, 15, 30, 45% of beetroot powder

Cupcake sample	Sensory attribute				
	Crust color	Crumb Color	Crumb Hardness	taste and flavor	Overall acceptability
Control sample	9±0.52	8.7±0.71	8.67±0.67	8±0.68	9±0.26
Beetroot (15%)	8.7±0.67	8.8±0.75	8.3±0.49	8.5±0.62	8.83±0.54
Beetroot (30%)	9±0.52	8.8±0.40	8.5±0.76	9±0.51	9.17±0.40
Beetroot (45%)	8±0.77	8.3±0.76	7.8±0.60	7.5±0.71	7.83±0.70

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## إنتاج كب كيك خالي من الجلوتين واللاكتوز مرتفع في محتواه من العناصر الغذائية ومضادات الأكسدة باستخدام مسحوق البنجر ولبن جوز الهند

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### المستخلص:

لمضادات الأكسدة دورا هاما وحيويا لدي المصابين بمرض السلياك، حيث أظهرت العديد من الدراسات زيادة فرصة حدوث الإجهاد التأكسدي لدي الأشخاص المصابين بهذا المرض، السبب الذي يؤدي إلي ضرورة إحتواء غذاء المرضى المصابين بالسلياك علي أطعمة غنية بمضادات الأكسدة لوقف تأثير فعل الشوارد الحرة الناتجة من زيادة تعرض الجسم للإجهاد التأكسدي، يعد البنجر وجوز الهند من الأغذية المعروفة والشائعة لدي العديد من الشعوب، حيث يحتوي كلا من البنجر وجوز الهند علي العديد من العناصر الغذائية الهامة للجسم، مضادات الأكسدة ومضادات الإلتهاب والتي يمكن أن تساعد مرضي السلياك في الوقاية من العديد من المضاعفات المصاحبة للمرض في حالة إستخدامها.

وقد هدفت الدراسة الحالية إلي دراسة تأثير إستبدال الدقيق الخالي من الجلوتين بمسحوق البنجر كمصدر للألياف، الأملاح المعدنية ومضادات الأكسدة بنسبة (0، 15، 30، 45%)، وكذلك إستبدال اللبن البقري (المحتوي علي اللاكتوز) بلبن جوز الهند، وذلك علي محتوى العناصر الغذائية والخواص الحسية في الكب كيك الخالي من الجلوتين واللاكتوز.

ولقد لوحظ من خلال نتائج الدراسة زيادة معنوية عند  $P \leq 0.01$  في الألياف، الرماد والرطوبة في جميع عينات الكب كيك المحتوي علي مسحوق البنجر ولبن جوز الهند، في نفس الوقت لوحظ إنخفاض معنوي في محتوى تلك العينات من الكربوهيدرات الكلية والدهون وكذلك في السرعات الحرارية وذلك مقارنة بعينات الكب كيك المعدة بالدقيق الخالي من الجلوتين.

كما وجدت النتائج زيادة معنوية في مستوي الماغنسيوم، الكالسيوم، الحديد، البوتاسيوم، الخارصين والصوديوم، كما لوحظ إرتفاع معنوي  $P \leq 0.01$  في مستوي المركبات الفينولية ومضادات الأكسدة في محتوى العينات المستخدم في إعدادها مسحوق البنجر ولبن جوز الهند، أظهرت النتائج الحسية أيضا أن إستبدال الدقيق الخالي من الجلوتين بمسحوق البنجر ولبن جوز الهند بنسبة 15 و 30% أدى إلي تحسين لون القشرة، الطعم والنكهة و ذلك أدى إلي تحسين مستوي القبول العام لعينات الكب كيك.