Production of high nutrients and antioxidans 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 and popular contain high amount of antioxidant and antiinflammatory properties, and could be an important assist in the treatment of many diseases**.**

This study aims to investigate the effect 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 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 is a gluten –responsive inflammatory disorder with a worledwide prevalence of 1%., celiac disease is a chronic inflammatory reaction which occurs in susceptible individuals in response to ingestion of gluten 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)***, Which found in wheate, rye and barley, is inherently challenging because gluten is ubiquitous, tasteless, and not always visible. Many patients with celiac disease eat gluten- free (GF) food prepared in a home kitchen alongside gluten- containing foods. Fear of gluten exposure is common among celiac disease patients and often leads to hypervigilance and decreased quality of life as reported by ***Wolf et al. (2018).***

More studies had shown correlation between celiac disease and increase in Reactive Oxygen Species (ROS) in the body which resulted in oxidative stress in people with celiac ***(Kashyap et al., 2022) .***

The over production of ROS occurs in patients with celiac disease due to consumption of gluten, thereby triggering a cascade of reactions that cause oxidative stress both in the small intestinal wall and the entire body. Oxidative stress can be blamed for free radical damage of significant cellular structures, thus adversely modifying their functions ***(Boda et al., 1999).***

Odetti et al. (1998) mentioned that individuals with untreated celiac disease has lower antioxidant potential which is perhaps a sign for an increased demand of antioxidants required to compensate the high amount of ROS produced in the body.

Rowick et al. (2018) reported that oxidative stress is a factor involved in the pathogenesis of celiac disease (CD), possibly affecting the course of the disease and celiac-related complications.

Efficient antioxidant mechanisms, both enzymatic and nonenzymatic, defend the body against free radical damage. Total antioxidant capacity (TAC) is an expression of a complete ability to neutralize free oxygen radicals that initiate oxidative damage as reported by ***(Spiteller, 2007 ; Stojiljković et al., 2009 and Nahla et al., 2018).***

At the same time, in the food industry, oxidation is a major cause of food quality changes that affect the nutritional qualities, the texture and the appearance of the food, leading to undesirable off-flavors and potentially toxic reaction products ***(Dong et Al., 2008).*** Thus, antioxidants play a vital role in both food systems and in the human body to reduce oxidative stress ***(Li et al., 2018)***.

The consumption of bakery products is increasing worldwide. Indeed, the cake is one of the most tasteful and appreciated bakery products which consumed by people of different ages ***(Douati et al., 2017)***. Their sensory quality, practicality, and convenience can be pointed as the major factors of the high consumption, since consumers are increasingly seeking easy-to-prepare meals that satisfy their sensory expectations ***(Manisha et al., 2012).***

Red beetroot (beet, garden beet, table beet) is a traditional and popular vegetable in many parts of the world. Red beetroot belongs to the *Beta vulgaris ssp. Vulgaris L.* subspecies, *Beta vulgaris L.* species, *Beta L.* genus, *Betoideae* subfamily of the goosefoot family (*Amaranthaceae*), and the *Caryophyllales* order ***(ceclu and Nistor,* 2020).**

Neelwarne and Halagur (2013) mentioned that the plants of the *Beta* genus are presumed to have originated in North Africa and spread through the Mediterranean Sea route, occupying the seashores of Europe, Asia and the Americas.

Ceclu and Nistor (2020) reported that a vegetable consumed worldwide due to its high content of biologically active substances, such as betalain, inorganic nitrates, polyphenols, folates, as well as its minerals and vitamins present in the tuberous root. The beet, like its cousin the turnip, is known for its edible leaves and roots. They are consumed in many ways, such as whole, cooked, canned or minimally processed products, depending on the region. Beetroot is used as a vegetable, and its juice and extracts also serve as traditional medicine, food colorant and additive to cosmetics. This plant has high antioxidant and antiinflammatory properties, and could be an important aid in the treatment of many diseases**.**

Also, beetroot are characterized to have medical effects as anti-inflammatory, antiallergenic, antiplatelet, antiatherogenic, cardioprotective, and vasodilatory ***(Balasundram et al., 2006).*** One of the important food quality parameters for attracting the consumers to edible products is color; it is called a consistency measure and is a necessary sensory trait for the approval of consumers. Many precautions of using synthetic dyes in manufacturing food products due to the health hazards, particularly in the initiation and development of chronic diseases as the transition in the histopathological function of the liver and kidney ***(Sahar and Manal, 2012)***.

Theba et al. (2020) reported that beetroot was extensively used as additives in the food industry because of its natural and harmless pigments and colorant properties and absence of toxicity. Beetroot powder has many functional properties like water holding capacity, water retention capacity, swelling capacity and oil absorption capacity so it can be widely utilized in different food products like cookies, cake, snacks, candies, etc. Utilization of beetroot pomace is inexpensive and it can be used as effective functional ingredient.

Coconut palm (*Cocos nucifera* L.) is an economic plant cultivated in tropical countries, mainly in the Asian region. It is very rich in fibre vitamins and minerals and has no gluten. Improves digestion, absorption of nutrients, vitamins and minerals. Coconut flour, a significant by product of coconut milk as reported by ***(Ramya and Anitha, 2020) & (Ramaswamy, 2014).***

Coconut milk is generally extracted from grated coconut meat after pressing or squeezing with or without the addition of water. Coconut milk has been used as a major ingredient for several cuisines such as curries and desserts ***(Tansakul and Chaisawang, 2006).*** Besides serving as a food ingredient.

Coconut milk is highly nutritious rich in vitamins and minerals as mentioned by ***(Vudugula and waghray, 2018)***. Also ***Alyaqoubi et al. (2015)*** Study of antioxidant activity and physicochemical properties of coconut milk. Results of the study showed that coconut milk samples exhibited a significantly different ($P<0.05$) antioxidant activity in comparison of goat and cow's milk for all the assays.

Thus, the objective of this research was to investigate the effect 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 purchased from local market of Suhag City, Egypt.

Preparation of coconut milk:

Coconut milk was prepared according method of ***Mepba et al., (2009)***. Meanwhile ground coconut meat was mixed with water in a ratio of 1:3 (w/v) coconut meat to water and the slurry filtered through double layers of cheese cloth.

Preparation of beetroot flour:

The dried beetroot were milled by house mincer (Moulinex, Super Blender, France). The beetroot flours were packed in polyethylene bags and kept until analysis use in the preparation of cupcakes.

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). After getting appropriate texture the dough was poured into paper cups and backed at 180˚C ± 5˚C for 12 - 20 min. The baked cupcakes were cooled down at room temperature in a sealed plastic bag then stored refrigerator at 5ºC to analysis.

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 oile 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:

Moisture, fat, crude protein, crude fiber and ash contents for all sample were determined as described in the ***AOAC (2010)***. Total carbohydrates was calculated by difference.

Determination of minerals:

The elements Ca, Fe, Zn and Mg were determined by ICP-Inductively Coupled Plasma Emission Spectrometer (iCAP 6200) according to ***(Isaac and Johnson, 2002)***. Na and K were determined using the Flam Photometer (Jenway PFP7) according to the procedure reported by ***(AOAC, 2005)***

Determination of total phenolics and antioxidant activity:

Total phenolic content was determined using Folin-Ciocalteu reagent according to ***Singleton and Rossi (1965),*** and The DPPH assay was done according to the method of ***Brand-Williams et al. (1995)*** with some modifications.

Sensorial evaluation of prepared gluten free cupcake:

All cupcake sample were cooled for 1-2 h at room temperature (25°C) in a sealed plastic bag. Sensory properties were evaluated for crust color, crumb color, crumb hardness, taste and flavor, overall acceptability**.**

Statistical analysis:

Data were analyzed using Statistical Package for Social Science (SPSS, 20), data were reported as mean ± 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,. Significance was declared at $P < 0.05$ and $P < 0.01$ ***(Pallant, 2005)***.

Results and discussion:

Nutritional indicators of prepared gluten free cupcake:

The data in Table (2) showed the chemical composition of cupcake substitution with different 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 is an abundant and good potential edible protein source, and demonstrated that the coconut cake protein fractions have potential usages in functional foods.

Dhawan and Sharma (2019) how revealed that beetroot flour is good source of carbohydrate, crude fibre, iron, calcium and vitamin C. Thus could be potentially be utilized as an ingredient in many dishes to enhance the nutritional value of food.

Alshehry (2019) evaluated the beetroot powder as chemical analysis, total phenolics, flavonoids compounds, antioxidant activity, minerals content and identification of betalains pigments by HPLC. Four concentrations of beetroot powder (2.5, 5.0, 7.5 and 10%) were substituted with wheat flour 72% extraction to prepare cupcakes, the results observed that the beetroot powder had contained rich source from crude fiber (20.4%), total phenolics, flavonoids compounds, and antioxidant activity. As well as the minerals content, potassium and sodium are the best sources in beetroot, and also, betalain was the highest amount pigment during identification of betalains pigment extracted 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 $^{**} P \leq 0.01$.

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 compered 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 contains a significant amount of phenolic acids: ferulic, protocatechuic, vanillic, p-coumaric, p-hydroxybenzoic and syringic acids, ***Yao et al. (2004)*** how reported that beetroots vegetable contains high amount of phenolic components, which can work as antioxidant and take part in prevention against tumour and cardio vascular disease.

Seneviranta and Dissanayake (2005) determined the phenolic antioxidant in coconut cake, found total phenolic content in coconut cake was 2156±16 mg/Kg. Also with ***(Mahfouz and Abd-Elnoor, 2017)*** how

found that beetroot are rich in phenolic compounds, flavonoids and antioxidant activity.

Ranawana et al. (2018) found that beetroot significantly improved the antioxidant and polyphenol profiles of sponge cake, and reported that beetroot improves the oxidative stability and shelf life of processed foods, and its effects could be enhanced through combining with other natural products.

Also agree with ***Theba, et al. (2020)*** who mentioned that beetroot contains higher amount of betalains and phytochemicals which can be utilized in food applications. Large amount of pomace are cheap source of dietary fibre, also contain various bioactive compounds and antioxidant activity.

Mistrianu et al. (2022) used antioxidant-rich beetroot peels powder (BPP) in different concentrations (1.5, 3, 5, and 7%) to obtain value-added mayonnaise. Found the BPP was characterized by a high betalain content (1.18 ± 0.03 mg/g DW) and rich polyphenolic content (225.36 ± 1.97 mg GAE/g DW(and showed high 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±0.35	2323.5±71.88
Beetroot (15%)	10.07**±0.23	3255.0**±55.43
Beetroot (30%)	11.46**±0.29	3375.0**±69.28
Beetroot (45%)	25.10**±0.05	16395.0**±25.98

Values are presented as means ± 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 are presented in Table (5). The results found no significant differences between 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 found blends of rice and coconut flour can be successfully incorporated into gluten free cookies, resulting in products with pleasant flavor and taste. Also reported that coconut flour possesses good nutritional properties which could be utilized for value addition of baked goods.

Ingle et al. (2017) found that the replacement of flour with beet roots powder by 10 % had a greater average color and appearance score. The ground color was darker as the amount of beet roots powder rose. Therefore, replacing up to 10 percent of wheat flour with beet roots powder led to excellent acceptability of cookies.

Also agree with **Alshehry (2019)** how found the substituted cupcake up to 10% from beetroot powder gives the best sensory characteristics, color (crust and crumb) and inhibition of bacteria and fungi and molds in cupcake until 10% substituted with beetroot.

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 بإستخدام 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 المرضي 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 والشائعه 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 محتوي 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 محتوي تلك العينات من الكربوهيدرات الكلية والدهون وكذلك في السعرات 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 محتوي 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% أدي إلي تحسين لون القشرة، الطعم والنكهة وكلك أدي إلي تحسين مستوي القبول العام لعينات الكب كيك.