Original Article

Cupcakes Preparation from Resistant Starch as Alternative Fat for Children and Treating Obesity in Mice

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Abstract

This study examined resistant starch at 10, 20, 30, 40 and 50% as an alternative fat with 10% chickpeas added separately to make cupcakes. Nutritional analysis of raw ingredients, sensory assessment, texture profile analysis (TPA), physical qualities of cupcakes and mouse obesity treatment with cupcake formulae examined. Chickpeas exhibited a greatest chemical composition and total phenolic and flavonoid concentrations at 28.75, 7.34, 5.74, and 7.32%. On dry weight, 265.35 mg/100 GAE and 104.46 mg/100g QE. Resistance starch had highest dry weight in insoluble and soluble dietary fiber at 38.79, 25.89, and 12.90%. As resistant starch grew, cupcake texture profile analysis increased. Acid, thiobarbituric, and peroxide acid values changed by how long resistant starch was stored and the amount used as a fat substitute. A biochemical investigation indicated that obese mice with 50% shortening substituted for fat had lower blood lipid profiles and estimated atherogenic indexes than controls but higher HDL cholesterol. Liver functions showed that groups fed on 10 to 50% resistant starch as a fat replacer in the obese rats' group decreased gradually from 35.14 to 25.37 mg/dl in ALP, from 15.66 to 7.94 mg/dl in AST and from 61.25 to 32.75 mg/dl in ALK. Up to 50% resistant starch as an alternative shortening in cupcake recipes was proven by antioxidant enzymes including GSH, SOD, CAT and MDA from obese rats. Resistance starch as a replacement fat until 50% alternative shortening and 10% chickpeas, which have high antioxidants as a dietary supplement to prevent and treat obesity-related disorders.

Keywords: Resistant starch, Alternative fat, Texture profile analysis, Cupcake formula

Introduction

The worldwide obesity epidemic is developing rapidly [1]. An increase in calorie consumption, poor physical activity, and our diets' nutritional composition contribute to this trend [2, 3]. In addition to public health policies and guidelines based on healthy diets like the Mediterranean diet, food reformulation, especially of processed foods like bakery goods and pastries, is crucial to fighting obesity [4]. To lessen the risk of chronic illnesses including cardiovascular disease, diabetes, and cancer, dietary reformulation must reduce calorie density, salt, added sugar, saturated and trans-fats [5].

Childhood obesity is a significant public health issue, impacting organ systems and causing psychological issues [6]. Currently, there are few pharmacological treatments, and the food industry is exploring healthy ingredients. Addressing childhood obesity is crucial to prevent adult obesity and metabolic and cardiovascular problems. Addressing juvenile obesity can lower morbidity, mortality rates, and hospital expenses in later life. To prevent personal and community health disasters, urgent corrective action is required to address the rapidly rising spread of childhood obesity. As a result, treating children is more difficult; even though childhood obesity is a major health issue [7]. The use of pharmaceutical or surgical therapies is strongly constrained by children's vulnerability.

Resistant starch (RS) is a dietary fiber and gut flora substrate, producing short-chain fatty acids (SCFA) through microbiota metabolism, providing energy to colonocytes and stimulating human metabolism [8].

RS has been investigated for its advantageous effects on controlling glucose homeostasis, enhancing gut health, and boosting satiety, all of which may have an impact on lowering obesity [9]. RS has positive benefits on obesity and associated disorders, primarily via controlling blood sugar and glycemic response, boosting fatty acid oxidation, reducing body fat formation, reducing hunger, and modifying gut microbiota composition [10].

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Chickpea (Cicer arietinum L), a popular legume, is known for its high protein content, antioxidants, antihypertensive, hypocholesterolemic, and anticancer properties, making it a valuable source of nutrients [11]. Although most studies focus on the impact of proteins, it has been demonstrated that their hydrolysates and alcoholic extracts also contain natural antioxidants such as phenolic and flavonoid compounds. As a result, their consumption is referred to as a replacement for the prevention of chronic degenerative diseases [12]. Due to their nutritional value and bioactive makeup, chickpeas play a significant role in the human diet. They are rich in fiber, fats, carbohydrates, and protein. Additionally, chickpeas include a variety of bioactive substances, important vitamins, and minerals [13].

The aim of this research therefore to investigate the combination of 72% wheat flour, chickpea, and resistant starch as alternative fat to obtain different formulas of protein-enriched cupcakes and high fiber to treat obese mice.

Materials and Methods

Materials

Chickpea (*Cicer arietinum* L.), Wheat flour (*Triticum aestivum L.*, 72% extraction), shortening (refined palm oil, 100% pure vegetable oil, and cholesterol free), sugar, eggs, salt, baking powder, dried skim milk, and vanilla, were obtained from the local market in Saudi Arabia.

Male albino mice (48 mice), whose weight ranged between 100-110 g and were obtained from Pharmacy College at King Saud University were used in this experiment. The basal diet was prepared according to AOAC and contained the following ingredients: 10% corn oil, 70% starch, 10% casein, 4% salt mixture, 1% vitamin mixture, and 5% cellulose [14].

Kits for determination of the parameters were purchased from Sigma-Aldrich Corp., MO, USA.

Methods

Preparation of Resistant Starch and Chickpea

Resistant starch was prepared by using corn starch weighed 200 g and was mixed with distilled water 700 ml and then autoclaved at 125°C for 1 hr. After that, it was cooled at room temperature and put overnight in a refrigerator at 4°C according to Po-Ying *et al*. [15].

Chickpea seeds were prepared by soaking them in water for 24 hr to decrease 53% of tannins and this treatment improves the digestibility of starch and reduces the content of protease inhibitors. Moreover, this phenomenon induces the leaching of soluble molecules such as monosaccharides, disaccharides, oligosaccharides, soluble polyphenols, and phytic acid [16]. The chickpea seeds were dried in an oven-air-dry and milled in a Wiley mill to give a fine powder.

Preparation of Cupcake

The cupcake processing method was taken according to Lebesi and Tzia [17]. The shortening (50.0 g), baking powder (5.0 g), dried skim milk (15.0 g), and vanilla (0.2 g) was mixed in a medium mixing bowl and the sugar (100 g) and salt (3.0 g) were added and mixing was continued for an additional 1 min. Eggs (44.0 g) were then added to the creamed mixture. Wheat flour (90.0 g) and a third of the dried mixture water were added and all were mixed for 45 seconds. The batter was poured into the aluminum cup. The cupcakes were baked in a 160°C oven for 45 min. Cupcakes were cooled to room temperature before use to give cupcake control

To prepare cupcakes that contained resistant starch as a fat replacer, the shortening in the cupcake's formula was replaced with 10, 20, 30, 40, and 50 % of resistant starch from shortening to give five formulas with low fat containing 45.0, 40.0, 35.0, 30.0 25.0 g shortening were added separately to Wheat flour plus chickpea (90.0 g plus 10.0 g). The same order of mixing described for the control was followed. After baking, all cupcakes were cooled at room temperature and kept in a refrigerator at 4 C until the experiments were performed.

Chemical Constituents of Raw Materials

Chemical analyses in 72%-extracted wheat flour, chickpea, and resistance starch were determined according to the method outlined in AOAC [14]. Total dietary fiber was estimated according to Prosky [18], whilst, soluble and insoluble dietary fibers were determined according to Lee and Prosky [19].

Qawasmeh *et al*. [20] used the Folin-Ciocalteu reagent to measure the total phenolic content (TPC) of the samples, and expressed the results as milligrams of gallic acid equivalents per 100 grams of dry weight. Similarly, Eghdami and Sadeghi [21] measured the flavonoid content of the samples and expressed the results as milligrams of quercetin equivalents per 100 grams of dry weight.

Sensory Evaluation of Cupcakes and Their Formula Fat Replacer

A 5-point hedonic scale was used to assess the sensory characteristics according to Rosa *et al*. [22]. The sensory evaluation was to estimate the variations between the control cupcake and their formula fat replacer. Twenty experienced staff members from the Family Education Department, Faculty of Education, Umm Al-Qura, Makka Al-Mukarama, Saudi Arabia were asked to judge the cupcakes and their formulae for appearance, texture color, taste, odor, and overall acceptance.

Instrumental Analyses of Cupcakes and Their Formula Fat Replacer

Texture Profile Analysis (TPA) indices of different cupcakes and their formula fat replacer were determined using a

Brookfield CT3 instrument (Brookfield Engineering Laboratories, Inc., MA 02346-1031, USA). From the force-time curve, it could be determined Firmness (N), gumminess (N), chewiness (N), adhesiveness (N.s), cohesiveness, springiness, and resilience were calculated according to Gomez *et al.* [23].

Extraction of Shortening from Cupcakes and Their Formula Fat Replacer

Shortening was exhaustively extracted from cupcakes and their formula fat replacer every week for four weeks. The extracts were filtrated, evaporated from the solvent, and kept in a freezer for analysis

Physical Analysis of Fat Replacer from Cupcakes and Their Formula During the Storage Period

Peroxide value (PV) and acid value (AV) were determined following AOAC [14], while thiobarbituric acid (TBA) value was measured following Egan *et al*. [24].

Biological Investigation

For seven days, fat and a base diet were supplied to experimental rats, which were then randomly divided into six groups of six rats each. The first major group, which was treated as control-negative rats, received a basic diet for an additional six weeks.

The seven mice groups induce obesity by feeding with a basal diet substituted with 20% fat from the corn oil and starch, a fatty basal diet. These groups were reclassified into control positive +(ve) as a group (2) was fed a fatty basal diet and the mixture of 20% from the control cupcake. Meanwhile, the rats of the 3^{ed}, 4th, 5th, 6th, and 7th groups were fed separately on a fatty basal diet and mixture of 20% from the cupcake and different formulae fat replacer at 10.0, 20, 30, 40, 50% from resistance starch as a fat alternative for six weeks experimental period.

After the experiment was complete, blood samples were obtained from the orbital plexus and centrifuged at 3000 rpm to get the sera. The sera were then stored at -20°C in a deep freezer until they were analyzed.

Triglycerides, total cholesterol, and high-density lipoprotein cholesterol (HDL-cholesterol) were estimated by Fossati and Principe [25], Allain *et al.* [26], and Lopez-Virelle *et al*. [27], respectively. Low-density lipoprotein cholesterol (LDL-cholesterol) was described by Wardlaw and Snook [28].

Liver functions as Alanine (ALT) and Aspartate (AST) transaminoferase were determined according to Reitman and Frankel [29]. Alkaline phosphate activity (ALk) was determined by Belfied and Goldberg [30].

Yoshioka *et al*. [31] used a calorimetric method to measure serum lipid peroxidation as malondialdehyde (MDA). Following Sairam *et al*. [32], the activity of the antioxidant enzyme superoxide dismutase (SOD) was measured in the serum, and glutathione (GSH), a non-enzyme antioxidant, was measured in the serum by Habig *et al*. [33]. Finally, the catalase enzyme (CAT) was measured according to Aebi [34].

Statistical Analysis

Analysis of variance (ANOVA) was used to assess the significance of the differences between the means of the variables. A significant difference ($p < 0.05$) was found in all variables. The data means test was used to compare the means of the samples in all analyses. All statistical analyses were performed using the SAS System for Windows software [35].

Results and Discussion

Chemical Constituents of Raw Materials

The results from the nutritional value of 72%-extracted wheat flour, chickpea, and resistance starch are obtained in **Table 1** which indicated that chickpea contained the highest in protein; total oils, ash content, and crude fiber were 28.75, 7.34, 5.74, and7.32% on dry weight, respectively, and the total carbohydrates were 50.85%. The data gathered by Wani and Kumar [36], who discovered that the moisture, protein, fat, ash, and fiber contents of chickpea powder were 6.34, 26.40, 6.20, 3.14, and 3.96 g/100g, respectively, are consistent with these findings. In the meantime, the dry weight percentages of the components of wheat flour were 12.48, 2.68, 0.54, and 0.81%, and the percentage of total carbohydrates was 83.48%. Protein (about 10%–12%) and starch (around 70%–75%) make up the majority of the ingredients in wheat flour, while polysaccharides (roughly 2-3%), lipids (roughly 2%), and total carbohydrates (roughly 80–90%) make up the minor ingredients [37]. Resistance starch, on the other hand, had the largest percentage of total carbohydrates 98.24% on dry weight despite having the lowest chemical analysis value.

Resistance starch had 38.79, 25.89, and 12.90% total, insoluble, and soluble dietary fiber on dry weight, followed by chickpea at 22.48, 16.12, and 6.36%. Wheat flour had the lowest dietary fiber fractions. Health benefits come from eating dietary fiber (DF), a category of carbohydrates that are hard to digest. It improves intestinal bolus size, accelerates intestinal transit, decreases colon cancer risk, and manages blood sugar [38]. Dietary fiber is soluble or insoluble. Soluble fiber includes gums, hemicelluloses, mucilages, and pectins, whereas insoluble fiber is mostly cellulose and lignins [39].

Chickpea seeds (265.35 mg/100 GAE and 104.46 mg/100g QE) had the highest total phenolic and flavonoid content, followed by wheat flour was 8.54 mg/100 GAE and 2.31 mg/100g QE, respectively. Total phenolic and flavonoid content was not detected in resistance starch. Conjugated mono-, di-, and oligosaccharides of phenolic chemicals, such as phenolic acids, tannins, and flavonoids, have been found

in the fiber structure. The biological characteristics of these changes vary; therefore fiber could be viewed as a natural source of antioxidants [40]. Numerous phenolic-rich products are produced by the food and agricultural industries. Secondary plant metabolites known as phenolic compounds are created during growth and reproduction. They provide crucial defense against a variety of infections and predators. Additionally, phenolic compounds have been observed to have anti-microbial, anti-inflammatory, and anti-allergenic characteristics [41].

Table 1. Chemical constituencies of raw materials

Chemical analysis	Wheat flour 72% extraction	Chickpea powder	Resistances starch
Protein	12.49±0.95	28.75±1.48	0.72±0.01
Lipid	2.68±0.04	7.34±0.42	0.09±0.01
Ash	0.54±0.01	5.74±0.14	0.50±0.01
Crude fibers	0.81±0.01	7.32±0.61	0.45±0.01
Total carbohydrates	83.48±4.52	50.85±3.28	98.24±8.16
TDF	3.17±0.04	22.48±1.49	39.56±2.43
ISDF	2.14±0.02	16.12±1.56	26.39±1.76
SDF	1.03±0.05	6.36±0.47	13.19±1.15
Total phenolic mg/100g gallic acid	8.54±0.02	265.35±8.25	-----
Total Flavonoids mg/100g quercetin	2.31±0.01	104.46±5.36	-----

Values (mean ±SD)

Sensory Evaluation of Cupcakes and Their Formula Fat Replacer

Sensory evaluation data for cupcake control and site formulae containing resistance starch as alternative fat are presented in **Table 2**. The resistant starch rising level had no appreciable impact on the descriptive evaluation metrics. Panelists reported no change in texture between the control cupcake and the cupcake containing 50 % RS and a decrease in taste for the cupcake containing 50% RS as alternative fat. These variations were not statistically significant because lipids absorb many flavor components, softening the flavor by lessening the sharpness of acid substances and contributing to flavor and tenderness, which is essential to the difference in taste [42]. Objective odor, color, and appearance measurements match sensory observations. After scoring at least 5, the judges approved all cupcake compositions for hedonic assessment. The 10% cupcake scored highest in acceptance. Despite a statistically significant difference between the RS and control samples, panelists preferred cupcakes up to 50% RS (25g resistance to 25g fat to create cupcakes). Majzoobi *et al*. [43] observed that adding 20% RS to wheat cakes did not affect their taste. RS may be added to cakes in bigger amounts, and the RS-containing cakes did not substantially vary from the control sample, providing nutritious fibers without affecting their taste.

Table 2. Sensory evaluation of cupcakes and their formula fat replacer

Formulae	Appearance	Texture	color	Taste	Odor	Overall acceptability
Control	4.75a±0.12	4.75a±0.12	4.75a±0.12	4.75a±0.12	4.75a±0.12	4.75a±0.12
Formula 10% Fat replacer	4.50ab±0.11	4.40ab±0.13	4.55ab±0.14	4.30b±0.16	4.45ab±0.11	4.44ab±0.14
Formula 20% Fat replacer	4.25ab±0.12	4.05b±0.11	4.35ab±0.16	3.85c±0.08	4.15b±0.09	4.13b±0.16
Formula 30% Fat replacer	4.00b±0.09	3.70c±0.09	4.15b±0.15	3.40cd±0.0,7	3.85c±0.07	3.82bc±0.08
Formula 40% Fat replacer	3.75c±0.08	3.35cd±0.07	3.95c±0.09	2.95d±0.06	3.55cd0±0.06	3.51c±0.06
Formula 50% Fat replacer	3.50c±0.06	3.00d±0.05	3.25d±0.07	2.50e±0.05	3.00d±0.05	3.05d±0.02

Values (mean ±SD) in the columns are statistically significantly different at ($P \leq 0.05$).

Instrumental Analyses of Cupcakes and Their Formula Fat Replacer

The texture profile analysis of the cupcake and their formula fat replacers are shown in **Table 3**. These characteristics are crucial since they demonstrate the product's quality and freshness. The greatest force needed to compress cupcake crumbs as they are constantly deforming is called firmness [44]. To determine the degree of tolerance in handling and packaging, cohesiveness quantifies the structure's stress [45]. Another essential characteristic of baked goods connected to an elastic and fresh aerated product is springiness, which is defined as the elasticity of the cupcake and its formula fat replacers [46].

The study found that the firmness of cupcakes increased from 9.86 N to 15.68 N in formula (5) 50% replacement fat due to higher addition of resistant starch, dietary fiber, and starch, which reduced gas storage capacity and mechanical strength. Additionally, water addition altered the cupcake's texture [47].

However, cohesiveness increased with the use of resistance starch cupcake formulae presented the highest value for this parameter than the control. When compared to the control, chewiness values which are directly proportional to firmness were more than twice as high in various cupcake formulations fat replacers. The high resistant starch level of this root was

present in all cupcake formulations, enabling the development of a robust polymeric matrix [48].

Comparing the cupcake formula (5) to the control cupcake, chewiness values (directly related to firmness) were greater in the cupcake formula (5) and included 50% fat replacer even more than twofold (5.40 vs. 16.25 N, respectively). All formulations included resistant starch, and adding dietary fibers to the mix enables the creation of a robust polymeric matrix and stability in the final goods [49].

Resistance starch fortification Chickpea-based cupcakes 10% enhanced springiness, resilience, gumminess, and adhesiveness from 0.75, 0.29, 6.82 N, and 0.54NS in cupcake control to 0.89, 0.54, 9.26N, and 3.85 NS in cupcake formula (5) with 50% resistant starch. Salazar *et al*. [49] say gumminess, adhesiveness, and springiness indicate stress resilience. Resilience and springiness show a product's deformation recovery. It shows the cake sample's adaptability. These features are usually assessed organoleptically by softly pressing a food item with their hands or lips and watching it expand back to its original size.

Table 3. Texture profile analysis of cupcake and their formula fat replacers

Formulae	Firmness (N)	Cohesiveness	Resilience	Springiness	Adhesiveness (N.S)	Chewiness (N)	Gumminess (N)
Control	9.86e±0.85	0.65d ±0.03	0.29d±0.01	0.75b ±0.06	0.54d ±0.04	5.40d±0.74	6.82d ±0.63
Formula 10% Fat replacer	10.35d±1.29	0.79d ±0.04	0.34c±0.03	0.77ab±0.06	1.21c ±0.07	7.53d±0.79	7.43c ±0.69
Formula 20% Fat replacer	11.86cd±1.31	0.95c±0.05	0.39c 0.03	0.79ab±0.05	1.87c ±0.09	9.66c±0.82	8.04b ±0.75
Formula 30% Fat replacer	12.94c±1.54	1.43b±0.17	0.45b±0.04	0.81a ±0.08	2.52b ±0.12	11.79b±0.84	8.54ab ±0.74
Formula 40% Fat replacer	14.37b±1.49	1.67ab±0.17	0.49b 0.06	0.84a ±0.07	3.19a ±0.17	13.92b±1.85	8.65b ±0.82
Formula 50% Fat replacer	15.68a±1.78	1.91a±0.19	0.54a±0.05	0.89a ±0.06	3.85a ±0.19	16.25a±1.83	9.26a±0.95

Values (mean ±SD) in the columns are statistically significantly different at ($P \leq 0.05$).

Physical Analysis of Fat Replacer from Cupcakes and Their Formula During the Storage Period

The acid value (AV), thiobarbituric acid value (TBA), and peroxide value (PV) of the cupcake control and its fat-replaced counterparts were measured during storage at room temperature and are presented in **Table 4**. The storage period and the concentration of resistance starch used as a fat replacer both significantly affected ($p \leq 0.05$) the AV, TBA, and PV. The cupcake control at zero time meant AV (1.12 mg KOH/ gm oil), TBA (0.22 mg malonldhyde/ kg sample), and PV (1.54 meq O2/ kg oil) and higher after four weeks were AV (8.61 mg KOH/ gm oil), TBA (1.34 mg malonldhyde/ kg oil), and PV (11.39 meq O2/ kg oil of active oxygen/ kg oil) than other cupcake control after four weeks were the highest AV (8.61 mg KOH/ gm oil), TBA (1.34 mg malonldhyde/ kg oil), and PV (11.39 meq O2/ kg oil of active oxygen/ kg oil). Whereas the cupcake formulae at 10, 20, 30, 40, and 50% fat replacer after four weeks were AV (7.78, 7.07, 6.31, 5.53, and 4.75 mg KOH/ gm oil), TBA (1.19, 1.04, 0.89, 0.47, and 0.59 mg malonldhyde/ kg oil), and PV (10.51, 8.91, 7.67, 6.43, and 5.19 meq O2/ kg oil of active oxygen/ kg oil) respectively.

The AV, TBA, and PV mean gradually ($p \leq 0.05$) decreased in all cupcake formulae with increasing the resistance starch as a fat replacer during the storage period for four weeks. These outcomes are consistent with according to Kozłowska *et al*. [50], the acid levels of the control cupcake were comparable after baking (1.12 mg KOH/g of fat), and the highest acid value was discovered for the control cupcake after four weeks. Additionally, the formulae were gradually reduced until the end of the storage period for all of the formulae after 28 days. According to Mahmoudi *et al.* [51], control cakes saw a greater increase in AV, TBA value, and PV during room-temperature storage than cupcake formulas with various concentrations of resistant starch and 10% chickpea.

Biological Investigation

Effect of Different Diet Resistance Starch Cupcakes on Lipids Profile in Rate

Serum lipid profiles for rats fed on a basal diet substituted with 20% cupcake control and its formula fat replacer at zero time and after the experiment which is shown in **Table 5**. Values of triglycerides, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol were significantly the same at the beginning of the experiment, which means good homogeneity of experimental units. Meanwhile, after the experiment, the outcome observed that the highest control negative was 268.94, 211.58, and 145.12 mg/dL, except the high-density lipoprotein cholesterol, which was the lowest at 50.64 mg/dL, respectively.

Concerning data from the end of the experimental period for the different groups' fat replacer, the triglycerides, total cholesterol, and high-density lipoprotein cholesterol were gradually decreased to 115.29, 100.93, and 85.19 mg/dL, whereas, the low-density lipoprotein cholesterol, which was

the lowest at 29.19 mg/dL, respectively in formula (5) which had contained 50% fat replacer. Moreover, the atherogenic index confirmed the lipid profile.

Table 4. Physical analysis of fat replacer from cupcakes and their formula during storage period

Cupcake formulae	Acid value (mg KOH/ gm oil)			Thiobarbituric acid value (mg malonaldehyde/ kg oil)			Peroxide value (meq O2/ kg oil)		
	Zero time	After 15 days	After 4 weeks	Zero time	After 15 days	After 4 weeks	Zero time	After 15 days	After 4 weeks
Control	1.12a ±0.11	4.48a ±0.43	8.61a ±0.95	0.22a ±0.02	0.69a ±0.61	1.34a ±0.12	1.54a ±0.15	8.87a ± 0.78	11.39a ±1.05
Formula 10% Fat replacer	1.12a ±0.11	4.16a ±0.41	7.84b ±0.84	0.22a ±0.02	0.65a ±0.54	1.19ab ±0.15	1.54a ±0.15	7.81ab ±0.72	10.15b ±0.97
Formula 20% Fat replacer	1.12a ±0.11	3.84b ±0.33	7.07b ± 0.75	0.22a ±0.02	0.59b ±0.48	1.04b ±0.12	1.54a ±0.15	6.75b ±0.54	8.91c ±0.86
Formula 30% Fat replacer	1.12a ±0.11	3.52b ±0.31	6.31bc ±0.54	0.22a ±0.02	0.54b ±0.43	0.89cb ±0.07	1.54a ±0.15	5.69c ±0.51	7.67d ±0.75
Formula 40% Fat replacer	1.12 ±0.11	3.21b ±0.34	5.53c ±0.43	0.22a ±0.02	0.49c ±0.42	0.74c ±0.05	1.54a ±0.15	4.63d ±0.46	6.43e ±0.63
Formula 50% Fat replacer	1.12a ±0.11	2.86c ±0.25	4.75d ±0.41	0.22a ±0.02	0.45c ±0.41	0.59d ±0.04	1.54 ±0.15	3.57e ±0.32	5.19f ±0.47

Values (mean ±SD) in the columns are statistically significantly different at ($P \leq 0.05$).

The serum total cholesterol is significantly reduced by the resistant starch. It effectively reduced plasma triglycerides and dramatically decreased plasma cholesterol. Consuming resistant starches decreases HDL cholesterol, which in turn prevents bile acids from being absorbed, increasing their excretion [52]. According to all of the findings, treating food with highly resistant starch can lower total cholesterol levels and raise HDL levels. The most crucial anti-atherogenic criterion is an increase in HDL. Additionally, a diet high in fiber can lower the atherogenic index (AI), and resistant starch shares many of the same physiological features as dietary fiber (RS) [53].

Table 5. Means of serum lipids profile in rats fed on different cupcake resistance starch

Groups	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Atherogenic index
Control negative	120.35e±10.15	98.74e±7.86	89.7a±5.21	25.0f±1.16	0.36
Control positive	268.94a±15.9	211.58a±15.32	50.64d±2.76	145.12a±12.35	1.11
Formula 10% Fat replacer	238.21ab±12.3	189.45ab±13.71	56.75cd±3.48	121.93b±10.21	1.08
Formula 20% Fat replacer	207.48b±10.5^{b}	167.32b±12.25	62.86c±5.25	98.74c±9.72	1.05
Formula 30% Fat replacer	176.75c±11.0^{b}	145.19c±11.36	68.97bc±5.89	75.55d±6.76	1.00
Formula 40% Fat replacer	146.02d±9.07^{c}	123.06d± 10.12	75.08b±6.27	52.36e± 4.25	0.91
Formula 50% Fat replacer	115.29e±9.68	100.93e±9.46	85.19a±5.19	29.19f±1.28	0.53

Values (mean ±SD) in the columns are statistically significantly different at ($P \leq 0.05$).

Effect of Different Diet Compositions on Serum Liver Functions

Liver functions as alanine (ALT) and aspartate (AST) transaminoferase, and alkaline phosphates (ALk) were determined in rat groups fed on different diets and the results are reported in **Table 6**. The results illustrated that higher activity in the enzyme ALT, AST, and ALK in control positive was 60.12, 17.59, and 69.25 mg/dl, respectively than in the control negative was 22.25, 7.16, and 30.12mg/dl, respectively. The reason for this large increase is hepatic injury, which causes these marker enzymes to leak out of the cytosol of the hepatocytes and into the bloodstream. This discovery is in line with that of Ghanbari *et al.* [53]. Hyperglycemia may cause liver cell fat increase and glycogen storage. This accumulation damages the liver and kidneys, raising hepatic enzyme levels, according to Julián *et al.* [54].

Whereas, the groups fed on 10 to 50% from resistant starch decreased gradually from 35.14 to25.37 mg/dl in ALP, from 15.66 to 7.94 mg/dl in AST, and from 61.25 to 32.75 mg/dl in ALK, respectively, these results showed that the different cupcake had contained rich amounts of resistant starch and natural antioxidant which status improve liver enzymes activity. A increasing body of studies shows that RS changes gut microbiota structure, enhances mucosal immunological tolerance, and promotes the production of microbial chemicals that directly stimulate intestinal epithelial cell proliferation. Dietary intervention with resistant starch may reduce diet-induced gut-liver axis dysfunction and fatty liver disease risk [55].

Table 6. Serum liver functions in rats fed on different cupcake resistance starch

Groups	ALT (mg/dl)	AST (mg/dl)	ALK (mg/dl)
Control negative	22.25^{d}± l.32	7.16^{d}± 0.82	30.12^{d}±2.56
Control positive	60.12^{a} ± 5.38	17.59^{a} ± 1.76	69.25^{a}±5.75
Formula 10% Fat replacer	53.14^{ab} ± 4.27	15.66^{ab}± 1.53	61.96^{ab}±5.34
Formula 20% Fat replacer	46.22 ^{b}± 3.62	13.73^{ab} ± 1.24	54.67^{b}±4.58
Formula 30% Fat replacer	39.27^{c} ± 2.59	11.80 ^{b}±0.97	47.38^{c}±3.71
Formula 40% Fat replacer	32.32^{c} ± 2.0047	9.87^{c}± 0.86	40.09^{c}±3.12
Formula 50% Fat replacer	25.37^{d}±1.75	7.94^{d}±0.84	32.75^{d}±2.89

Values (mean ±SD) in the columns are statistically significantly different at ($P \leq 0.05$).

Effect of Cupcakes and Their Formula Fat Replacer on Oxidative Stress of Rats

The antioxidant enzymes and the malondialdehyde (MDA) were determined in different rat groups treated with resistance starch as a fat replacer and compared with the control mice health group and the results are reported in **Table 7**. The results showed that the mice control positive was the lowest in GSH, SOD, and CAT, as well as MDA by 9.57, 4.69, and 3.78 U/L, and also, MDA, was the highest by 330.49 nmol/ml, respectively. The mice control negative group was the highest in all antioxidant enzymes (18.24, 12.96, and 8.12 U/L), and the lipid peroxidation as malondialdehyde (MDA) was the lowest by 175.52 nmol/ml.

The rats' groups fed on cupcakes containing 10, 20, 30, 40, and 50% from resistance starch as a fat replacer gradually increased the antioxidant defense, that is, enzymes glutathione (GSH) from 11.13 to 17.37 U/L, superoxide dismutase (SOD) from 6.16 to 12.52 U/L, and catalase (CAT) from 4.60 to 7.89 U/L, respectively, whereas, a significant decrease in lipid peroxidation as malondialdehyde (MDA) from 298.52 to 170.65 nmol/ml. Moreover, the results reported that the cupcake made different concentrations of resistant starch and 10 % chickpea which contained high amounts of flavonoids and phenolic acid. These findings supported the Chen *et al.* [55] finding that antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) proteins are elevated by polyphenols. Free oxygen radicals and a decline in antioxidant factors may be to blame for the pulmonary damage brought on by a high level of lipid peroxidation. Malondialdehyde (MDA) levels in rat lungs were found to be higher than normal, and this resulted in lower levels of both enzymatic and non-enzymatic antioxidants as well as significant DNA damage, according to earlier studies [56].

Table 7. Influence of cupcakes and their formula fat replacer on oxidative stress of rats

Groups	GSH (U/L)	SOD (U/L)	CAT (U/L)	MDA (nmol/ml)
Control negative	18.24^{a}±1.51	12.96 ^{a}±1.12	8.12^{a}±0.72	175.52 ^{d}± 11.82
Control positive	9.57^{e}±0.87	4.69 ^{e}±0.42	3.78 ^{e}±0.05	330.49 ^{a}± 15.431
Formula 10% Fat replacer	11.13^{d}±0.96	6.16 ^{d}±0.51	4.60 ^{d}±0.06	298.52^{ab}± 13.39
Formula 20% Fat replacer	12.69^{c}±1.05	7.75^{c}±0. 62	5.42 ^{c}±0.03	266.55 ^{ab}±13.76
Formula 30% Fat replacer	14.25 ^{b}±1.39	9.34 ^{b}±0.75	6.24^{b}±0.04	234.58 ^{b}±13.58
Formula 40% Fat replacer	15.81^{ab}±1.43	10.93^{ab}±0.94	7.06^{a}±0.13	202.61^{c}±12.43
Formula 50% Fat replacer	17.37^{a}±1.147	12.52^{a}±1.05	7.89^{a}±0.15	170.65 ^{d}±11.46

Values (mean ±SD) in the columns are statistically significantly different at ($P \leq 0.05$).

Conclusion

Finally, it could be concluded that the cupcake which contained 10 to 50 % resistance starch as replacing shortening and separately added 10% chickpea which contained high phenolic and flavonoid content was found without significant effect on the organoleptic properties and corresponding

consumer acceptance. These formulae were fed on the mice obese different groups and the results observed that the lipid profile, liver function, and antioxidant enzymes were decreased until 50% resistance starch replacing shortening.

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