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Development of Fortified Biscuit with Sweet and Acidic Whey Proteins and Its Effects on Body Weight, Indices of Body Composition, and Antioxidant Capacity in Rats

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ABSTRACT

The effect of sweet (SW) and acid whey (AW) proteins on body weight (BW), composition (BC), and antioxidant capacity (AC) is uncertain. SW and AW proteins obtained from local white cheese and labneh production lines were dehydrated, developed biscuits containing these protein powders and evaluated biscuits' sensory attributes and proximate nutrient contents, and investigated their effects on BW, indices of BC, and AC in rats. Four types of biscuits were prepared: plain (PB), albumin (ALB), SWB, and AWB. Five isocaloric and isonitrogenous diets containing Albumin (control), PB, ALB, SWB, and AWB were used. Sixty adult male Sprague-Dawley rats were used, 10 were sacrificed at the start, and the remainder were randomly assigned to the five diet groups (10 rats/ group) and fed for four weeks. BW, food intake (FI) and water intake (WI), food efficiency ratio (FER), serum AC, liver weight (LW), Lee index (LI), body mass index (BMI), length, and abdomen and chest circumferences were measured following standard protocols. AW was higher in ash and fat whereas lower in carbohydrates but similar to SW in protein and moisture contents. ALB had the highest protein content, AWB had the most increased fat and ash contents, and SWB had the highest moisture content. PB had the highest carbohydrates and the lowest ash contents among the other biscuits. PB and SWB were the best in all sensory attributes tested and the overall acceptability compared to other biscuits. The four biscuit diets kept similar BW, whereas the control diet kept the highest BMI, LI, and LW, which was also not significantly different from the initial group. Animals fed the PB diet had the most increased length, abdomen, and Chest Circumference, whereas these measurements were lowest in animals fed SWB and AWB diets which were not different from the control. Animals fed the AWB diet had the lowest WI (535.012 ml), but all other diets were not significantly different from the control diet in FI and FER. Serum AC was the highest in animals fed SWB and AWB which were insignificantly different from the initial group, whereas it was the lowest in PB and ALB. Given their sensory attributes and effects on AC and BW status, SW and AW may be used as protein substitutes in bakery products; however, further studies are warranted.

Keywords: Sweet whey, Acidic whey, Proximate nutrients, Body weight, Body composition, Antioxidant capacity.

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INTRODUCTION

Whey protein, a by-product of the dairy industry, causes a profound environmental problem due to its slow degradation rate that can reach the surface of groundwater and soil (Veskouki *et al.,* 2020). Whey protein is a compound with a strong antioxidant effect because it is rich in cysteine residues that are vital for the synthesis of glutathione *in vivo* and the improvement of the antioxidant profile in the liver, small intestine, lung, and muscle (Veskouki *et al.,* 2020).

Muscular weakness is a phenotype of many clinical conditions associated with impaired movement and increased mortality (Hakim *et al.,* 2013). Loss of muscle mass and function exacerbates many health outcomes, such as metabolic disorders, particularly diabetes mellitus (Camargoa *et al.,* 2020). Some studies have shown that whey protein, combined with resistance training, can improve muscle performance by stimulating protein synthesis, a protective factor against muscle weakness (Hakim *et al.,* 2013; Bell *et al.,* 2017).

Body composition is often assessed for determining body component deficiencies or excesses, such as lean mass and fat mass, which allow an understanding of nutritional status. Accurate methods for determining animal body composition are critical for understanding how the body responds to nutrient intake and for metabolic and physiological studies (Angeloco *et al.,* 2012). Abdominal obesity results from many metabolic disorders; thus, it is crucial to assess and track the changes time in the fat mass (Bruce and Byrne, 2009). Whey protein may regulate food intake through several body mechanisms (Patel, 2015). Some indices of body composition may identify obesity and predict its adverse effects on lipid profile and oxidative stress in animals (Rothwell and Stock, 1981). It has been found that alterations in body mass index (BMI) are associated with dyslipidemia profile and oxidative stress in the serum of rats; therefore, BMI may predict these adverse consequences of obesity in rats (Novelli *et al.,* 2007).

The two kinds of whey, sweet and acidic, produced by the cheese and yogurt industries, respectively, are considered by-products (Abdelhakam *et al*., 2022). They could have a different composition of amino acids (Muuronen *et al.,* 2020). Acid whey is the liquid drained when dairy producers make yogurt or soft cheese after casein precipitation by acidification of lactic acid bacteria, leaving whey proteins in soluble form (Carvalho *et al.,* 2012). Sweet whey is the liquid that is leftover when dairy producers make hard cheese by rennet enzyme action on the casein, leaving the whey proteins also in soluble form. Compared to the former, the latter contains a higher level of lactose, as it escapes degradation by the lactic acid bacteria, therefore called sweet whey (Carvalho *et al.,* 2012). To our knowledge, no studies are available that investigate and compare the effect of the inclusion of the two types of whey proteins in the diet on body weight, body composition, and antioxidant capacity. Thus, the objectives of the present study were to dehydrate sweet whey and acidic whey proteins extracted from local white cheese and labneh production lines, develop biscuits containing these protein powders evaluate biscuits' sensory attributes and proximate nutrient contents, and investigate the effects of such biscuits on body weight, indices of body composition, and serum antioxidant capacity in rats.

MATERIALS AND METHODS

Whey collection and drying

Two kinds of whey, sweet (SW) and acidic (AW) were collected from the production lines of the local cheese and yogurt, respectively, at the pilot dairy processing plant of the Department of Nutrition and Food Technology, at the University of Jordan. The collected whey was kept refrigerated at ~5 °C for not more than two days until the drying process.

The two types of whey were dried by a drum dryer (R. Simon LTD Nottingham, England). The drum dryer was operated at 140ºC at the drum speed of 20 rpm. Corn starch was added at the level of 2% (w/w) prior (within 30 min) to drying to prevent whey overheating and burning. The dried particles were collected by scrappers located at the end of each cycle of the rotating drums, in which the collected particles were considered to have reached complete drying. To avoid atmospheric moisture

capture, the dried particles were vacuum packaged in thick polyethylene packages and kept refrigerated at ~5 °C for no more than one month.

Biscuit preparation and inclusion of the whey

Plain biscuits (PB) were prepared from raw ingredients listed in Table (1). The preparation procedure for the PB biscuits was as follows: using the standard measuring cup, dry ingredients were mixed (flour, baking powder, and salt) until homogeneity. In another dish, the whole egg, sugar, and butter were mixed by whisking them, and then they were added to the above mixture and mixed until homogeneity. Water was gradually added to the mixture and kneaded until a continuous Dough was formed. Whey dried particles (acidic and sweet) and albumin (Hen's egg white crystals medium (Food Ingredients Europe, 2022) were introduced into PB biscuits at the level of 15% (w/w) to produce three types of treated biscuits, SWB, AWB, and ALB, respectively as shown in Table (1). Pieces of the dough were mold shaped and were set onto butter paper, then baked in a thermally controlled electrical oven (Combi -Zanussi) at 200ºC for 10 min equipped with an electrical fan for uniform distribution of heat over the biscuit's pieces therefore uniform baking.

Table 1: Ingredients used to prepare the plain and fortified biscuits

Ingredients	Measure	Amount (g)
Flour	1cups	240
Protein source (whole egg, sweet whey, acidic whey, or albumin)	½ cup	120
Butter /animal source	---	100
Sugar	¼ cup	60
Baking powder	½ tsp	2.5
Salt	½ tsp	2.5
Water	6 tbsp/ 2tbsp plain biscuits	90 g /30 g plain biscuits
Total	---	615 g /555g

Source: www.kica-academy.com

The proximate analysis of whey and biscuits

The SW and AW powders and the four biscuits preparations were analyzed chemically following the proximate analysis technique for the determination of protein, fat, moisture, ash, and carbohydrate according to the AOAC procedures (AOAC, 1995). Total protein content was determined by the Kjeldahl method, based on the determination of the total nitrogen concentration using the Dumas' method of Nitrogen analyzer Vario Max cube (Elementar Analysensysteme GmbH, Langenselbold, Germany). Fat content was determined by the soxhlet extraction procedure using the Roses-Gottlieb method (AOAC, 1995). Ash content was determined by burning off the organic matter in a Muffle furnace at 550 °C-650 °C for 24 hrs, then determining the residual weight representing the inorganic matter content (AOAC, 1995). The moisture content was determined by oven–drying procedure using a specialized oven (Axier Ltd type: TH200, Novasina instrument, Switzerland) operated at 105 °C for 3h. Total soluble carbohydrates were calculated by the difference between the sample weight minus the total weight of other determined ingredients (moisture, protein, fat, and ash).

Sensory evaluation of biscuits

The AWB, SWB, PB, and ALB were tested for sensory attributes using the hedonic test (Munoz, 2013), which includes a nine-point hedonic scale ranging from (9) Like extremely to (1) Dislike extremely. The evaluation was performed in two sessions (two replicates) with the same 15 semi-trained, randomly chosen students

and professors from the Department of Nutrition and Food Technology at the University of Jordan in each session. The students were chosen randomly from both genders, and the testing procedure was performed in a specialized sensory analysis laboratory. Samples were presented on four separate plates. Drinking water was used to wash mouth sensory buds between the samples. The evaluators were asked to provide their opinions regarding the color (gentral, homogenty), flavor, smell,crispiness, glossiness and overall acceptability.

Animal Experimentation

Diet mixture preparation

Five isonitrogenous and isocaloric diets were prepared and assigned as albumin (control), ALB, PB, AWB, and SWB diets. Experimental diet mixtures were formulated according to Reeves (1997). The basic chemical composition of these diets is summarized in Tables (2 and 3). The experimental diet mixtures were prepared to contain the same amount of protein, fat, carbohydrate, vitamins, and mineral elements but different in the source of the protein (SWB and AWB), while it was the whole egg in PB and ALB diets. The proximate nutrient contents of the four types of biscuits were considered in the diet formulation. Diet mixtures were freshly prepared daily, well-packed in air-free low-density polyethylene bags, and stored refrigerated at ~5 °C for no more than 24h of use.

Table 2. Macronutrient and energy content of the experimental diets.

Parameter	Control	ALB	PB	SWB	AWB
Energy (kcal/100g)	424	424	424	424	424
Carbohydrate (%)	65.1	65.1	65.1	65.1	65.1
Protein (%)	14.0	14.0	14.0	14.0	14.0
Fat (%)	12.0	12.0	12.0	12.0	12.0

Control: albumin diet, ALB: albumin biscuit diet, PB: plain biscuit diet, SWB: sweet whey biscuit diet, AWB: acidic whey biscuit diet.

Table 3: The basic ingredient composition of the experimental diets (g/kg).

Ingredient	Albumin Diet (Control)	Plain Biscuit Diet	Albumin Biscuit Diet	Sweet Whey Biscuit Diet	Acidic Whey Biscuit Diet
Egg albumin	140	110	80	125	125
Cornstarch	550.1	318.1	309.1	330.1	333.1
Sucrose	100	100	100	100	100
Corn oil	120	14	31	2	0
Plain Biscuit	0	397	0	0	0
Albumin Biscuit	0	0	421	0	0
Sweet Biscuit	0	0	0	436	0
Acidic Biscuit	0	0	0	0	412
Fiber- Wheat bran	50	50	50	50	50
Mineral mix	35	35	35	35	35
Vitamin mix	10	10	10	10	10
Choline bitartrate	2.5	2.5	2.5	2.5	2.5
TBHQ	0.008	0.008	0.008	0.008	0.008

Energy (Kcal)	4240	4240	4240	4240	4240

Based on Reeves (1997).

The animal study

Adult male Sprague Dawley rats (200-250g) were used as an animal model in a randomized control design experiment. Rats (n=60) were obtained from the experimental animal unit at Jordan University of Science and Technology, Irbid, Jordan. After an adaptation period of 1 week, 10 animals were sacrificed; plasma and serum were obtained and kept frozen at -20 °C till analyzed. Rats (n=50) were then randomly divided into 5 main groups (n=10/ group) and fed the diets given in the above table 3 (Control, PB, ALB, SWB, AWB diets) for 4 weeks. At the end of the experimental period, rats were fasted for 8 hours and anesthetized by chloroform. Blood was collected by cardiac puncture in plain tubes, centrifuged to obtain serum, and the liver was also removed; both the liver and serum were kept frozen at -20°C till analysis.

Experimental diets and water were provided *ad libitum* during the course of the study. Rats were individually housed in a well-ventilated room with a constant temperature of 25°C and a 12-hour dark/12-hour light cycle (Al-Badarein and Ahmad, 2020). Body weight, food intake, and water consumption were measured once a week. All the experiments involving animals were approved by the Institutional Animal Ethics Committee and carried out according to the recommended guidelines for animal care and use (NRC, 2011).

Following standard protocols (Novelli *et al.,* 2007), for each rat in the control and experimental groups, and a day before experimental termination, body length was measured from the nostril to the base of the tail (pelvic-caudal junction). Abdominal circumference at the point immediately anterior to the forefoot and chest circumference at the site immediately behind the foreleg were recorded. Body mass index (BMI) was calculated by dividing the animal's weight (g) by the square of its length (cm). The Lee index was determined by dividing the cube root of the body weight (g) by the nose-to-anus length (cm).

Serum total antioxidant capacity

Serum samples were analyzed in duplicates for total antioxidant status by a standard kit-based ELISA procedure (Geno Chem- SL1402Ra, Valencia, Spain). The kit measures the ferric-reducing ability of serum spectrophotometrically at 450 nm. The biochemical analyses were conducted in the Chemical, and Food Laboratories of the Jordan Food and Drug Administration (JFDA, Amman, Jordan). The protocol of each analysis was followed as instructed in the commercial biochemical kits′ leaflet (the manufacturer's instructions).

Statistical analysis

Data obtained in the animal study were analyzed using SAS statistical program. Results were analyzed using one-way ANOVA, and values were carried out in duplicate and presented as means ± SEM. Means were separated using Duncan's Multiple Range test, and levels of significance were tested at $P \leq 0.05$. Pearson correlation coefficients were used to assess different biomarkers in this study.

RESULTS AND DISCUSSION

Preliminary experiments in whey drying process

Different parameters were tested concerning the drying process using a drum dryer. These parameters included temperature, drum speed, and corn starch addition level. Regarding the addition of corn starch, was used to prevent lactose overheating and caramelization and the stickiness of the dried whey on the dryer drums. Samples without starch addition were found to have a dark brown color. The addition of hydrated and pre-gelatinized starch resulted in a substantial delay in the drying process. This is because water bound to starch particles is added to the total moisture in the starch-whey mixture, therefore, requiring higher energy to evaporate (Wootton and Bamunuarachchi, 1978). When the native (powder) starch was added, it absorbed moisture from the whey and then got gelatinized by the heat of drying. Thus,

this bound water prevents lactose caramelization and burning.

Regarding the effect of drying temperature, it was found that 150ºC produced whey powder with a brownish color, indicating lactose caramelization and/ or Millard reaction, which was prevented at 140 ºC. Therefore, a temperature of 140ºC was chosen for drying. Regarding the drum speed, it was observed that the speed of 20 rpm (the highest speed available) provides whey flakes with light yellowish color, but at the drum speed of 15 rpm, the product became dark again, therefore, 20 rpm was chosen.

According to the USDEC standards (USDEC, 2011), the optimum color of sweet and acidic whey powders varies from off-white to creamy. Since lactose is a reducing sugar, exposing the whey (particularly sweet whey) to high temperatures during drying may result in browning by the Millard reaction, while excessive heating could lead to sugar caramelization and burning. This colorization may become more obvious during improper storage (high temperature and relative humidity) conditions of whey powders (Dattatreya *et al.,* 2007). The addition of corn starch was successfully used in whey drying to prevent overheating (Jack and Wasson, 1940). Yousef *et al* (1997) dried sweet and acid whey using a hot surface rotary plate using a mixture composed of 16:3:1 of liquid sweet whey: wheat flour: and corn starch, respectively. The resulting powder had acceptable color, good flow ability, and storage stability. Therefore, these drying conditions were chosen to be used in addition to starch addition at the level of 2 and 4% to sweet and acidic whey, respectively.

Chemical composition of the whey powder

Protein, fat, carbohydrates, moisture, and ash are presented in Table (4). Results show that sweet whey powder has lower fat and ash but higher carbohydrate content than acidic whey, while both are similar in protein and moisture content. The Reasons for the higher fat content of acidic whey than sweet whey could be related to better emulsification and dissolution properties of lactic acid (which is more hydrophobic) than with sweet whey. The amount of fat in the whey is affected by many factors like seasonal changes, whey source and composition, cheese manufacturing conditions, and techniques used for evaluation contents of whey (Regester and Smithers, 1991, Schmidt *et al*., 1984; Abdelhakam *et al*., 2022).

The ash content in the current study is within the range reported by other researchers. JSMO (2008) dictated that the ash content should not be higher than 8.5% (w/w), and according to the USDEC (2011), the ash content of sweet whey powder ranges between 8.2-8.8%. Sawyer (2010) studied the ash content in sweet whey powder and found it to be 8.27%. Mustafa *et al.,* (2014) reported that the ash content in the produced sweet whey sample was 5.4%. which agrees with our results. It was concluded that the minerals in whey are only a small part of the liquid whey and are overshadowed by the whey's larger constituents of lactose, casein, whey proteins ,and water. The ash content of acidic whey powder is about three times that of sweet whey powder. The principal minerals in whey are potassium, calcium, chloride, phosphorus, and sodium, although sweet whey is expected to contain a higher salt content. It was reported that the salt content (NaCl) in sweet whey powder was found to be 2.7% (Cashman, 2006). This could be attributed to the source of this salt that is coming from the addition of sodium chloride to the cheese during processing this explains the reason sweet whey has a higher ash content as compared with acid whey powder. Whey contains approximately 10% salts of the dry matter, either as a natural component originating from the milk, or salts added during cheese processing ($CaCl_2$ and/or NaCl). In another study, the NaCl content in sweet whey powder was found to be from 2.5 to 4.0% (El-Desoki, 2009).

The high levels of fat together with a higher level of added starch could have contributed to increasing the ash content in the acidic whey. It was reported that adding starch to the sweet whey at a level of 2.5% and to the acidic whey at a level of 5% increased the total solid in the liquid from about 7 and 9%, respectively. It was reported that whey contains various minerals, the principal minerals in whey are potassium, calcium, chloride, phosphorus, and sodium (Cashman, 2006).

Table 4: Proximate analysis of sweet and acidic whey powder produced by drum drying with theaddition of 2% or 4% (w/w) starch to sweet or acidic whey, respectively.

Analysis	Sweet whey powder	Acidic whey powder
Protein %	17.9±0.002^{A}	18.45±0.003^{A}
Fat %	3.61±0.012^{B}	7.11±0.02^{A}
CHO%	69.27±0.051^{A}	69.95±0.05^{A}
Ash%	5.22±0.011^{B}	2.16±0.087^{A}
Moisture %	4.00±0.021^{A}	2.33±0.011^{A}
Total	100	100

Values are mean ± SD

Values in the same row with different superscripts are significantly difference ($p \leq 0.05$).

Regarding the level of carbohydrates (lactose), it is similar to those reported by other researchers (Table 4). Mustafa *et al* (2014) reported that the lactose content of fresh acidic whey is about 44.4%. Glass and Hedrick (1976) determined lactose content in commercially produced fresh sweet and acid whey collected from 12 to 15 dairy plants at monthly intervals for a year and found that their content was 69.4 and 63.2%, respectively. Guo et al (2010) reported that lactose in the dried sweet whey (produced by spray dryer procedure) was >70.5% (w/w). According to the Jordanian standards for sweet whey powder, the lactose content should be no more than 65% (JSMO, 2008).

As shown in Table (4), the protein content of whey powders was 18.45% and 17.9% in acidic and sweet whey, respectively, but there was no significant difference between them. Baer *et al* (1983) found that the protein content of sweet and acid whey powder ranged from 9-11%. According to JSMO (2008), the protein content of sweet whey powder should not be less than 10%. Glass and Hedrick (1976) showed that total protein, 13.0% ,and 11.7%, for sweet- and acid-type whey, while the non-protein nitrogen was 0.50 and 0.58%, respectively. Lactose and protein concentrations in the spray-dried powder were 72.5 396 ± 1.7% and 9.8 ± 0.2%, respectively, and agree with our study. The level of protein in whey powders in the current study was within the range mentioned previously. However, the amount of protein in the whey is affected by many factors like seasonal changes that could affect α-lactalbumin, β-lactalbumin, glycol macro peptide, and casein contents of whey (Regester and Smithers, 1991). Many other factors affect the protein content, like whey source and composition, cheese manufacturing conditions, the moisture content in the raw milk, heat treatment conditions, storage conditions, overall sanitation conditions, and techniques used for functionality evaluation (Schmidt *et al.,* 1984; Abdelhakam *et al*., 2022). Carbohydrate contents in both whey powders are not significantly different at $p \leq 0.05$. Moisture content is not significantly different between both whey powders at $p \leq 0.05$ and agrees with JSMO (2018), that moisture content should not be more than 5% (w/w)and 4.5% (w/w) in sweet and acidic whey powders respectively.

Chemical composition of biscuits

Results of table 5 show that the ALB had the highest protein content than other biscuits. This result is expected as ALB has albumin, which contains the highest protein content than the other biscuits containing SWB and AWB (Table 4). Fat content varied significantly ($P<0.05$) between biscuits, where the lowest is for ALB but not significantly ($P \geq 0.05$) different among the other treatments. This finding may be because albumin in the ALB is free of fats whereas, the containment of the whole egg, sweet whey, and acidic whey in biscuits (Table 1), which contain fat (Table 3), contributes in addition to this component to the PB, SWB, and AWB, respectively.

Table 5: Proximate nutrient analysis of produced biscuits.

Biscuits	Protein %	Fat %	CHO%	Ash %	Moisture %
Plain biscuits	7.51±0.02^{b}	26.65±0.03^{b}	58.54±0.01^{a}	2.30±0.00^{d}	5.00±0.01^{c}
Albumin biscuits	14.15±0.0^{a}	21.15±0.00^{c}	57.14±0.02^{b}	3.60±0.00^{b}	3.96±0.00^{d}
Sweet whey biscuits	3.50±0.00^{c}	27.00±0.03^{b}	50.53±0.10^{d}	2.84±0.00^{c}	16.13±0.07^{a}
Acidic whey biscuits	3.60±0.00^{c}	29.01±0.04^{a}	52.72±0.06^{c}	4.87±0.00^{a}	9.80±0.008^{b}

Values are mean (g/100g) ± SD (n=3 for each treatment group).

Values with the same column with different superscripts are significantly difference ($p \leq 0.05$).

Rostamia *et al* (2020) showed that the nutritional composition of a biscuit serving 50 g is 26.60% of energy protein, 30.01% of energy fat, and 40.18 % of energy carbohydrate. Biscuits were fortified with whey protein isolate (Rostamia *et al.,* 2020) and were analyzed for protein (Kjeldahl digestion), fat (Soxhlet method), saturated fatty acid, carbohydrate, and sugar content (Fehling method) using AOAC procedures (AOAC, 1995). Our results do not agree with those of Rostamia *et al. (*2020) study; this could be due to different analytical methods, biscuit composition, and study protocols involving fortified biscuits with whey protein.

Sensory evaluation of biscuits

As shown in Table (6), the lowest scores for color in general, color homogeneity, flavor, smell, crispness, and overall acceptability were for ALB and AWB, which had around 4 (dislike slightly) scores, whereas PB and SWB had significantly ($p \leq 0.05$) higher scores. The SWB got the best scores, which did not differ significantly from the control (PB). The reason for this finding could be due to the contribution of lactose sugar that is present in sweet whey. The sugar provides sweetness for flavor and undergoes the caramelization process while oven backing, a matter which also adds to the acceptable flavor, smell, crispness, and even glossiness; Therefore, for general acceptance (Tsakali *et al.,* 2010). The high protein content in the ALB (Table 4) could have increased the biscuits' glossiness by a denatured protein produced by heating. ALB and AWB had the lowest sensory scores (except glossiness for ALB) for attributes evaluated than the PB, which could be related to the lower fat content in the ALB (table 8) and the acidic taste of AWB associated with the presence of lactic acid.

Table 6: Sensory Evaluation of Produced Biscuits using Hedonic Scale*

Attribute / Biscuit	Overall Acceptability	General Color	Color homogeneity	Flavor	Smell	Crispness	Glossiness
Plain	6.40±0.45^{a}	6.10±0.44^{a}	5.96±0.41^{a}	6.06±0.43^{a}	5.40±0.53^{a}	5.86±0.60^{a}	6.56±0.50^{a}
Albumin	4.33±0.63^{b}	4.06±0.55^{b}	4.63±0.55^{b}	3.86±0.58^{b}	4.63±0.58^{b}	4.10±0.58^{b}	5.43±0.61^{a}
Sweet whey	6.36±0.49^{a}	5.83±0.54^{a}	5.86±0.51^{a}	6.20±0.47^{a}	5.90±0.51^{a}	5.43±0.57^{a}	5.33±0.62^{a}
Acidic whey	4.66±0.56^{b}	4.46±0.53^{b}	4.70±0.58^{b}	4.50±0.43^{b}	4.46±0.53^{b}	4.13±0.50^{b}	4.00±0.66^{b}

Mean ± SEM (n=15).

Values in the same column with different letters are significantly different ($p \leq 0.05$).

*1: dislike extremely, 2: dislike very much, 3: dislike moderately, 4: dislike slightly, 5: neither like or dislike, 6: like slightly, 7: like moderately, 8: like very much, 9: like extremely.

Body weight and indices of body composition

Table (7) shows the initial body weight, final body weight, changes in body weight, BMI, lee index, liver weight, and relative liver weight of rats fed experimental diets for four weeks. The initial and final body weights (after four weeks) body weight change of the four groups are not significantly (P≥0.05) different from each other and the control. BMI was significantly lower for the PB and ALB than the initial group, control group, SWB, and AWB diet groups. This result could be due to the lower protein content in these diets, which is related to the lower content of protein (as egg albumin) component (Table 3). Even though the ALB contained the highest protein content than other biscuits (Table 5), the level of addition to the diet (421g/kg) did not contribute to increasing the protein content. The PB had significantly lower protein content than the ALB (Table 5). Therefore, the produced diet with 397 g/kg (the lowest amount of biscuit addition among the diets) (Table 6) is expected to contain lower protein.

Table 7. Body weight and indices of body composition of rats fed experimental diets for four weeks.

Variable / Diet Group	Initial body weight(g)	Final body weight (g)	Body weight change (g)	BMI	Lee Index	Relative Liver weight %	Liver weight (g)
Initial group	135.120± 2.691[b]	NA	NA	0.132± 0.002[a]	0.147± 0.001[a]	3.761± 0.001[a]	5.111± 0.283[b]
Control	173.201± 4.492[a]	260.011± 5.911[a]	90.501± 4.911[a]	0.137± 0.001[a]	0.149± 0.000[a]	2.860± 0.002[b]	7.572± 0.330[a]
Plain	174.012± 4.981[a]	266.902± 5.471[a]	92.902± 5.201[a]	0.129± 0.001[b]	0.143± 0.001[a]	2.941± 0.002[b]	7.953± 0.270[a]
Albumin	175.201± 4.902[a]	264.012± 6.832[a]	88.802± 5.322[a]	0.126± 0.001[b]	0.145± 0.000[a]	3.053± 0.002[b]	8.161± 0.350a
Sweet whey	176.011± 4.151[a]	256.402± 7.583[a]	80.101± 5.851[a]	0.134± 0.002[a]	0.148± 0.000[a]	3.102± 0.030[b]	8.092± 0.430[a]
Acidic whey	175.120± 4.821[a]	268.521± 6.981[a]	93.501± 7.982[a]	0.136± 0.003[a]	0.149± 0.0012[a]	3.111± 0.002[b]	8.453± 0.360[a]

Mean ±SEM (n=10 for each group).

Values in the same column with different superscripts are significantly different (p≤0.05).

BMI: body mass index=weight (g)/length (cm^2).

Lee index; cube root of weight (g) divided by length (cm).

Relative liver weight =liver weight g/body weight g*100.

There is no significant (P>0.05) effect of the diet types on the liver weight and relative liver weight (Table 7). Therefore, the four Lee Index was not significantly (P≥0.05) different between the initial group, control, and other groups. This finding may indicate that there is no obesity occurring in all groups. Novelli *et al* (2007) tested the hypothesis that the anthropometrical index may identify obesity and may predict its adverse effects on lipid profile and oxidative stress in rats. They concluded that the BMI for male adult Wistar rats ranged between 0.45 and 0.68 g/cm2, and obesity may be easily estimated from the BMI in rats. Alterations in BMI were associated with a dyslipidemic profile and oxidative stress in the serum of rats, and BMI may predict these adverse consequences of obesity in rats.

Dietary biscuit groups did not affect liver growth performance in Sprague Dawley male adult rats during four weeks of experimental regimes. This result could indicate that apparently, there were no abnormalities in the growth performance in treated groups which are not significantly different from each other but significantly different from the initial group (Lee *et al.,* 2006). It was demonstrated that when type 2 diabetic NSY/Hos mice were fed an AIN76-modified high-fat diet supplemented with 1% (w/w) kaki-tannin for eight weeks, they kept similar kidney weights. This finding may indicate the little effect of diet on liver weight (Matsumoto and Yokoyama, 2012). Similar results were found by Zimmermann *et al* (2013), where thirty adult male Wistar rats were used to study the effect of barley extract addition on the antioxidant potential in rats and liver weight. It was shown that there were no significant differences in the liver weight of treated groups compared with control ones (Zimmermann *et al.,* 2013). The results of the current study are not consistent with those of Lee *et al* (2006). Lee *et al* (2006) demonstrated that the relative weights of the liver were significantly lower in the group treated with permission leaf extract compared to the high-fat control group. Furthermore, differences in experimental design, animal models, diet composition, sources, and forms may account for the differences between the current results and those of others (Lee *et al.,* 2006).

Table (8) shows no significant differences in weight between the four types of biscuit groups and between them and the control group, which was significantly ($P<0.05$) the lowest. The length and circumstances of the abdomen and chest were significantly the highest in PB and ALB-fed groups compared to the control and the other groups. Also, there was a significant difference ($p\leq0.05$) in length between the PB and ALB (Avilés-Santa *et al.,* 2017). Abdominal circumference, lee index, and BMI correlated significantly with body composition. This positive correlation between carcass fat and BMI (Avilés-Santa *et al.,* 2017) agrees with that of Novelli *et al (2007),* who suggested that BMI can reliably estimate body fat in rats even though it is not sensitive enough to detect body changes stemming from diets with different macronutrient compositions. Contrary to the present experiment, the cited study did not show the data regarding the correlation between carcass fat and the lee index and abdominal circumference. Carcass fat was significantly associated with BMI, Lee index, and abdominal circumference, suggesting that these parameters may be used for estimating rat body composition (Avilés-Santa *et al.,* 2017).

Table 8: Anthropometrical measures of rats fed experimental diets for four weeks.

Diet group	Weight (g)	Length (cm)	Abdomen circumstance (cm)	Chest circumstance (cm)
Initial group	135.12±2.69^{b}	34.75±0.41^{d}	16.13±0.48^{d}	12.13±0.48^{d}
Control	260.10±5.93^{a}	44.11±0.26^{c}	23.30±0.20^{b}	19.51±0.15^{c}
Plain	266.91±5.47^{a}	46.71±0.14^{a}	24.51±0.15^{a}	21.01±0.13^{a}
Albumin	264.01±6.83^{a}	45.41±0.35^{b}	25.52±0.47^{a}	20.31±0.61^{a}
Sweet whey	256.41±7.60^{a}	43.42±0.40^{c}	21.51±0.32^{c}	19.00±0.54^{c}
Acidic whey	268.51±6.98^{a}	43.90±0.49^{c}	23.32±0.37^{b}	19.11±0.29^{c}

Mean ±SEM (n=10 for each group).

Values in the same column with different superscripts are significantly different ($p \leq 0.05$).

Gerbaix *et al* (2010) showed central fat mass from the whole body DXA scan (extending from L2 to L5 vertebrae) correlated strongly with ex-vivo fat mass ($r = 0.94$, $p < 0.001$). Abdominal circumference was associated significantly with ex-vivo fat mass ($r = 0.82$, $p < 0.001$) and central fat mass (0.90, $p < 0.001$) in the whole group of rats. When dividing the overall group into lean and fat rats, correlations remained significant

between central fat mass and ex-vivo fat mass, but it disappeared for the lean group, between the abdominal circumference and ex-vivo fat mass (Gerbaix *et al.,* 2010).

Table 9 shows the accumulative food intake, food efficiency ratio, and water intake for PB, ALB, SWB, and AWB-treated groups and control. There are no significant differences (P≥0.05) between the four-type biscuits group and the control group in the three parameters that were recorded, but the water intake was significantly the highest in AWB compared to other groups and the control. The reason behind this result could be the acid whey coagulum created when milk is acidified by *lactobacillus* culture or mineral acid at a maximum pH of 5.1; thus, whey composition and sensory characteristics may vary depending on the kind of whey (acid, sweet) and the source of milk, cow or sheep or bovine (Tsakali *et al.,* 2010). So, the preparation method of acid whey and its milk source may be the reason for the acid composition, making rats thirsty.

Table 9: Food (FI) and water intakes (WI), food efficiency ratio (FER), and serum total antioxidant capacity of rats fed experimental diets for four weeks.

Diet group	FI (g)	FER	WI (ml)	Serum total antioxidant capacity (OD: 450nm)
Initial	---	---	---	0.241±0.000[a]
Control	701.811±12.332[a]	0.130±0.021[a]	606.501±27.481[b]	0.212±0.005[b]
Plain	717.131±5.190[a]	0.139±0.021[a]	628.011±24.720[b]	0.211±0.006[b]
Albumin	692.382±6.370[a]	0.127±0.024[a]	535.013±24.720[b]	0.197±0.006[b]
Sweet whey	683.641±8.340[a]	0.117±0.027[a]	575.012±27.920[b]	0.239±0.006[a]
Acidic whey	702.142±12.330[a]	0.133±0.035[a]	806.011±47.750[a]	0.235±0.006[a]

Mean ±SEM (n=10 for each group)

Values in the same column with different superscripts are significantly different ($p<0.05$).

Food intake: grams of accumulative food intake throughout a four-week experiment.

Food Efficiency Ratio = body weight change (g)/food intake (g).

Water intake: Accumulative water intake of rats throughout the four-week experiment.

OD: Optical density.

Veskouki *et al.* (2020) found no statistically significant differences in body weight, food, and water intake, and organ weight between the experimental groups (n= six rats) fed a standard commercial diet plus sheep/goat whey protein in a dose equal to 1 g/kg of body weight/day dissolved in drinking water for 28 consecutive days, and also between these experimental groups and the control group fed a standard commercial diet only. In another study, feeding male C57BL/6J mice of 6–8 weeks of age coleb leaf aqueous extract for 20 days led to no significant alterations in water intake between the control and experimental groups (Jadeja *et al.,* 2011).

The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron transfer that produces a violet solution in ethanol. In vitro, the DPPH assay is a reliable method for measuring total antioxidant activity (Kedare and Singh, 2011). The optical density value is proportional to the TAOS concentration in the sample. The higher levels of antioxidant activity are represented by lower radicals in the biological system. As shown in Table (9), both SWB

and AWB had significantly ($p \leq 0.05$) higher antioxidant activity than the control, PB, and ALB.

Novelli *et al* (2007) demonstrated that body weight gain, feed efficiency, and serum oxidative stress were attributed to obesity in male Wistar rats fed a control chow and monitored for up to 150 days of age. The authors reported similar results in male Wistar rats given control chow, and control chow with drinking 30% sucrose or a high-carbohydrate diet (Novelli *et al.,* 2007). They also showed that food consumption, energy intake, and body weight increased with age while the specific rate of body weight gain was significantly decreased (Novelli *et al.,* 2007). There was no significant difference between rat groups in body length and thoracic circumference (Novelli *et al.,* 2007). The abdominal circumference and body mass index significantly increased with enhancing age (Novelli *et al.,* 2007). There were positive correlations between body mass index and fat and length. They concluded that the body mass index in male adult Wistar rats ranged between 0.45 and 0.68 g/cm2 and was directly related to obesity, which, in turn, was associated with serum oxidative stress (Novelli *et al.,* 2007).

Veskouki *et al.* (2020) found that whey protein improved the antioxidant profile of the liver, small intestine, lug, and muscle, whereas it did not affect the redox state of the kidney. These results were indicated by tissue-specific alterations in the protein expression of glutamate cysteine ligase, catalase, glutathione S transferase, and superoxide dismutase-1. Although tissue-specific, it is articulated that the action of whey protein is biologically beneficial and could serve as a biofunctional constituent for foods improving body-redox profile when administered against redox-related diseases (Veskouki *et al.,* 2020). In another study, whey protein acted as a biofunctional component that enhances the antioxidant status of rats (Kerasioti *et al.,* 2018). Whey protein is a mixture with potent antioxidant action since it is rich in cysteine residues, which are necessary for glutathione synthesis in vivo (Veskouki *et al.,* 2020).

Conclusions

In conclusion, the results of the current study support that whey protein enhances serum antioxidant capacity and positively impacts body weight and its composition indices. The findings also support the use of whey protein as a substitute for protein in bakery products, especially sweet whey, as its sensory acceptability. Thus, whey protein proves to be a functional food rich in antioxidant capabilities and body weight control. Furthermore, the present data approve the inclusion of whey protein as a feed source for animals.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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تطوير بسكويت مدعم ببروتينات الشرش الحلو والحامضي وتأثيره على وزن الجسم ومؤشرات تكوين الجسم والقدرة المضادة للأكسدة في الجرذان

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ملخص

إن تأثير بروتين الشرش الحلو (SW) والحامضي (AW) على وزن الجسم (BW) وتكوينه(BC) والقدرة المضادة للأكسدة (AC) غير مؤكد. قمنا بتجفيف بروتينات SW وAW التي تم الحصول عليها من خطوط إنتاج الجبن الأبيض واللبنة، وطورنا البسكويت الذي يحتوي على مساحيق هذه البروتينات، وقمنا بتقييم الصفات الحسية للبسكويت ومحتوياته من العناصر الغذائية التقريبية، ودرسنا تأثيرها علىBW ومؤشراتBC وAC في الجرذان. وتم تحضير أربعة أنواع من البسكويت: سادة(PB) البيومين(ALB) وSWB وAWB. وتم استخدام خمسة خلطات غذائية متساوية للبسكويت في محتواها من الطاقة والنيتروجين وتحتوي على الألبيومين (الضابط)، وPB وALB وSWB و. AWB وتم استخدام ستين من ذكور جرذان-Sprague Dawley، وتم التضحية بعشرة جرذان في البداية، وتم تعيين الباقي بشكل عشوائي لمجموعات النظام الغذائي الخمس (10 جرذان/ مجموعة) وتم إطعامهم لمدة أربعة أسابيع. وتم قياس كل من BWوتناول الطعام (FI) والماء (WI) ونسبة كفاءة الغذاء (FER) وAC مصل الدم ووزن الكبد (LW) ومؤشرلي (LI) ومؤشر كتلة الجسم (BMI) والطول ومحيط البطن ومحيط الصدر باتباع البروتوكولات القياسية. وكان AW أعلى في الرماد والدهن بينما كان أقل في الكربوهيدرات ولكنه مماثل لـ SW في محتوى البروتين والرطوبة. وكان ALB يحتوي على أعلى محتوى من البروتين، وكان AWB يحتوي على أعلى محتوى من الدهن والرماد، فيما كان SWB يحتوي على أعلى محتوى رطوبة. واحتوى PBعلى أعلى نسبة كربوهيدرات وأقل محتوى رماد بين أنواع البسكويت الأخرى. وكان PB وSWB الأفضل في جميع الصفات الحسية التي تم اختبارها والقبول العام مقارنة بالبسكويت الآخر. وحافظت خلطات البسكويت الأربعة على نفس الوزن، بينما حافظت خلطة الضابط على أعلى مؤشر كتلة جسم وLI وLW والذي لم يكن أيضًا مختلفًا بشكل كبير عن المجموعة الأولية. وكانت الحيوانات التي تتغذى على خلطة PB هي الأكثر زيادة في الطول ومحيط البطن ومحيط الصدر، بينما كانت هذه القياسات أقل في الحيوانات التي تغذت على خلطة SWB وAWB والتي لم تكن مختلفة عن المجموعة الضابطة. وكانت الحيوانات التي تتغذى على خلطة AWB تتمتع بأقل نسبة) WI 535.102 مل)، لكن جميع الخلطات الغذائية الأخرى لم تكن مختلفة بشكل كبير عن الخلطة الضابطة في FI وFER. وكانت AC هي الأعلى في الحيوانات التي تتغذى على خلطة SWB وAWB والتي كانت مختلفة بشكل طفيف عن المجموعة الأولية، بينما كانت الأقل في خلطات PB وALB. ونظرًا لخصائصها الحسية وتأثيراتها على AC وBW، فإنه من الممكن استخدام SW وAW كبدائل بروتين في المخبوزات؛ ومع ذلك، فهناك ما يبرر إجراء مزيد من الدراسات.

الكلمات الدالة: الشرش الحلو، الشرش الحامضي، العناصر الغذائية التقريبية، وزن الجسم، تكوين الجسم، القدرة المضادة للأكسدة.

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