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Characterization of protein-enriched yogurt and its effects on the lean body weight gain and electrical activity in skeletal muscle of physically active individuals

Caracterização de iogurte enriquecido com proteínas e seus efeitos no ganho de peso corporal magro e na atividade elétrica no músculo esquelético de indivíduos fisicamente ativos

Caracterización del yogur enriquecido con proteínas y sus efectos sobre el aumento de peso corporal magro y la actividad eléctrica en el músculo esquelético de individuos físicamente activos

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Abstract

Protein-enriched yogurts have experienced a growth in marketability in recent years, being demanded especially by consumers interested in increasing their daily apportion of proteins. In this study, the objective was to develop a protein-enriched yogurt and to compare it with a non-enriched yogurt in terms of their physicochemical characteristics, texture, and sensory acceptance after different storage periods and their effects on the lean body weight gain and electrical activity in skeletal muscle of physically active individuals. The supplemented whey protein had a buffering effect on the product, leading to its lower syneresis. Both formulations had similar hardness, indicating that the textural parameters had been retained in the protein-enriched yogurt. Panelists indicated no differences in sensorial attributes between the non-enriched and protein-enriched yogurts, with the sensory characteristics of each product being mainly described by the presence of whey protein and the curdled texture. Consumption of the protein-enriched yogurt did not influence the lean body weight gain by physically active individuals.

Keywords: Dairy products; Product development; Nutrition.

Resumo

Os iogurtes enriquecidos com proteínas têm experimentado um crescimento em sua comercialização nos últimos anos, sendo solicitados principalmente por consumidores interessados em aumentar sua proporção diária de proteínas. Neste estudo, o objetivo foi desenvolver um iogurte enriquecido com proteínas e compará-lo com um iogurte não enriquecido em termos de suas características físico-químicas, textura e aceitação sensorial após diferentes períodos de armazenamento e seus efeitos no ganho de peso corporal magro e atividade elétrica no músculo esquelético de indivíduos fisicamente ativos. A proteína de soro de leite suplementada teve um efeito tamponante sobre o produto, levando à sua sinérese mais baixa. Ambas as formulações tiveram dureza semelhante, indicando que os parâmetros texturais foram mantidos no iogurte enriquecido com proteína. Os avaliadores não indicaram diferenças nos atributos sensoriais entre os iogurtes não enriquecidos e os enriquecidos com proteínas, sendo as características sensoriais de cada produto descritas principalmente pela presença da proteína do soro e pela textura coalhada. O consumo do iogurte enriquecido com proteínas não influenciou no ganho de peso corporal magro em indivíduos fisicamente ativos.

Palavras-chave: Produtos lácteos; Desenvolvimento de produto; Nutrição.

Resumen

Los yogures enriquecidos con proteínas han experimentado un crecimiento en su comercialización en los últimos años, siendo demandados especialmente por los consumidores interesados en aumentar su proporción diaria de proteínas. En este estudio, el objetivo era desarrollar un yogur enriquecido con proteínas y compararlo con un yogur no enriquecido en términos de sus características fisicoquímicas, textura y aceptación sensorial después de diferentes períodos de almacenamiento y sus efectos sobre el aumento de peso corporal magro y la actividad eléctrica en el músculo esquelético. de individuos físicamente activos. La proteína de suero suplementada tuvo un efecto amortiguador sobre el producto, lo que provocó una menor sinéresis. Ambas formulaciones tenían una dureza similar, lo que indica que los parámetros de textura se habían conservado en el yogur enriquecido con proteínas. Los panelistas indicaron que no hay diferencias en los atributos sensoriales entre los yogures enriquecidos con proteínas y no enriquecidos, y las características sensoriales de cada producto se describen principalmente por la presencia de proteína de suero y la textura cuajada. El consumo de yogur enriquecido con proteínas no influyó en el aumento de peso corporal magro de los individuos físicamente activos.

Palabras clave: Productos lácteos; Desarrollo de produtos; Nutrición.

1. Introduction

Yogurt, a fermented dairy product, is very popular among consumers of various age groups and socioeconomic status, especially for its health benefits. To have high consumer acceptance, plain yogurt has to have a smooth, uniform, and spoonable texture, without lumps, graininess, and visual whey separation (Lucey; Singh, 1997), besides a clean and typical yogurt flavor (Jorgensen et al., 2019). In recent years, yogurts with high protein contents have been highlighted in the dairy market, and the consumption of such protein-enriched yogurts has increased. Milk proteins, which are made up of casein (77%–78%) and whey proteins (17–18%), are important components of dairy products. Aside from their good nutritional benefits, milk proteins also convey functional properties to the milk products, such as gelation, texturization, emulsification, foaming ability, and flavor binding (Lesme et al., 2020). Accordingly, they are added to a wide range of food products as functional ingredients.

The proteins present in or added to foods have several health benefits. An increase in dietary protein can help in dietary strategies for weight loss and in preventing weight regain following weight loss. Protein also has a role in appetite control and satiety (Douglas et al, 2013; Morell et al., 2015) and in reducing blood lipids, blood pressure, and insulin resistance in overweight and obese individuals (Pal et al., 2010). There are many different types of whey powder available on the market; namely, whey protein concentrates, whey powder isolates, or whey powder hydrolase. These have different characteristics depending on the processing technique applied before the drying step, such as demineralization, lactose removal, whey protein concentrate supplementation, or straightforward drying.

Yogurt is defined as a fermented dairy product derived from the fermentation of milk by two species of bacterial cultures, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, which can be followed, in a complementary way, other lactic acid bacteria that, by their activity, contribute to the determination of the characteristics of the final product (Brasil, 2007). According to Codex standards, yogurt should contain a minimum of 2.7% milk protein and less than 15% fat (World Health Organization & Food and Agriculture Organization of the United Nations, 2011). However, there is currently no legal standard for defining "high-protein yogurt" (Jorgensen et al., 2019), with the generally accepted levels being a minimum of 5.6% protein and less than 15% fat. Higher levels of protein can be attained by fortification with milk powder, evaporation, or membrane filtration methods prior to fermentation, or by draining, mechanical separation, or membrane filtration methods after fermentation (Jorgensen et al., 2019).

The purpose of the present study was to develop a yogurt product with a high level of protein. Subsequently, its physicochemical and textural characteristics and sensory acceptance after different storage periods were assessed as well as its effects on the lean body weight gain and electrical activity in skeletal muscle of physically active individuals.

2. Methodology

2.1. Yogurt formulation

Reconstituted skim milk powder (15%, w/v) was used to obtain two yogurt formulations: non-enriched yogurt (control) and protein-enriched yogurt. Whey protein concentrate was added at 25% (w/v) to the protein-enriched product. Stevia (0.15%, w/v) and vanilla essence (0.20%, w/v) were added to both formulations. The mixtures were first pasteurized at 65 °C for 30 min and then cooled to 42 °C, and finally 0.01% (w/v) yogurt culture (YF-L812, Christian Hansen, Valinhos, Brazil) was added. Immediately thereafter, 50-mL aliquots of the mixture were placed into individual plastic packages and left to ferment for 4 h at 42 °C, following which the products were cooled to 4 °C. The yogurts were stored at 4 °C until analysis at days 1, 7, 14, 21, 28, and 35 of storage. The two formulations were produced three times.

2.2. Chemical analysis

The protein content was determined by measuring the total nitrogen in the products, using the Kjeldahl method and a conversion factor of 6.38. The ash content was determined gravimetrically (AOAC, 2012). The fat content was determined using the Gerber method (Brasil, 1981) and the lactose content was determined with the methylamine method (Lawrence, 1968), using a spectrophotometer (600 Plus, Femto, São Paulo, Brazil).

The color parameters (L*, a*, b*) were measured using a colorimeter (model CR-400, Konica Minolta, Osaka, Japan). The pH was measured at ambient temperature with a pH meter (Tec-3MP, Tecnal, Piracicaba, Brazil) and the titratable acidity was determined using the Dornic method (IAL, 2008). The composition and color of the formulations were evaluated at day 1 of storage. All chemical measurements were carried out in triplicate.

2.3. Syneresis

Yogurt samples were centrifuged at 1000 × *g* for 10 min at 8 °C (model 5804, Eppendorf, Hamburg, Germany). The separated whey was then carefully decanted using a pipette and weighed, and the centrifuge tube was also weighed to confirm removal of the whey. Syneresis was calculated as the weight of the whey separated per unit weight of yogurt (Aichinger et al., 2003). The degree of syneresis was expressed as a percentage.

2.4. Viability of yogurt bacteria

The enumeration of viable bacteria in the yogurt was carried out using the plate count method (Sifuentes dos Santos et al., 2019). For *Lactobacillus bulgaricus*, samples were spread onto MRS agar plates (Acumedia, Lansing, MI, USA) with the pH adjusted to 5.4 and incubated under anaerobic conditions for 72 h at 37 °C. For the enumeration of *Streptococcus* *thermophilus*, samples were spread onto M17 agar plates (Sigma-Aldrich, Buchs, Switzerland) and incubated aerobically for 48 h at 37 °C. Data are presented as the base 10 logarithm of colony-forming units per gram of sample (Log CFU g^{-1}).

The products were also analyzed for the presence of thermotolerant coliforms, coagulase-positive staphylococci, *Salmonella* spp., and *Listeria monocytogenes*, as required by local legislation (Brasil, 2001).

2.5. Texture profile analysis

Five yogurt sample replicates were used for the texture profile analysis, which was conducted using a Brookfield texture analyzer (CT3, Brookfield Engineering Labs, Middleboro, MA, USA) with a TA3/1000 cylindrical probe, a penetration speed of 1 mm/s, a distance target of 5 mm, and a trigger of 15 g (Buriti et al., 2008). The samples were taken out of the refrigerator just before the test operation. The hardness, adhesiveness, cohesiveness, and gumminess values were obtained. For each sample, the syneresis analysis, test for viability of the yogurt culture, and texture profile analysis were all performed at each cold storage period described in section 2.1 above.

2.6. Sensory analysis

The sensory analysis was carried out by 96 untrained panelists (18–60 years old, of both genders) recruited from the staff and students of Unopar University. None of the individuals presented intolerance or allergy to milk components. The sensory sessions were performed in individual testing booths under white light. Approximately 25-g portions of the two yogurt formulations were served in a random order in disposable transparent polyethylene cups coded with randomized 3-digit numbers. The test was carried out under controlled conditions, with mineral water and cream crackers made available to the panelists. Information about each participant's consumption of yogurt and proteic yogurt and practice of physical activity was obtained. Each panelist used a 9-point hedonic scale (1 = extremely disliked; 5 = neither liked nor disliked; 9 = extremely liked) to rate the appearance, flavor, texture, and overall acceptance of each product. They were also asked to indicate their intention of purchase of the product, using a verbal numeric scale ranging from 1 to 5 (1 = I certainly would not buy; 3 = Maybe I would buy, maybe not; 5 = I would certainly buy). The final evaluation was a check-all-that-apply (CATA) questionnaire, with 13 sensory (acid, aftertaste, artificial flavor, bitter, curdled aspect, curdled texture, liquid, presence of bubbles, presence of whey, sweet, sweetener flavor, vanilla flavor, yellow color) and 7 non-sensory terms (It is great for supplementation; It is a good breakfast choice; It is a healthy option; It is good for gaining lean mass; It is a nutritious food; It is perfect for after sport; This product gives me satiety). The participants were instructed to check all the attributes that applied to the yogurt they tasted. The order in which the attributes were presented was randomized within the two groups (sensory and non-sensory). This study was approved by the Ethics Committee of the University North of Paraná (Register 2.805.239/2018), and consent forms indicating voluntary and fully informed participation were signed by all participants.

2.7. Lean body weight gain

A double-blind randomized study was conducted to evaluate the lean body weight gain from consumption of the protein-enriched yogurt. The inclusion criteria were individuals who practice weight training or Muay Thai, were 18–30 years old, and were conducting physical activity for more than 6 months and no longer than 24 months. The exclusion criteria were individuals with any pathology related to obesity or renal pathology. Of 320 individuals who were approached at two academies of Londrina municipality (Brazil) that offered weight training and Muay Thai classes, 60 demonstrated interest in participating, and of those, 21 scheduled an anamnesis session. Finally, 16 attended the first day of the study and were enrolled. These individuals were blinded and randomly divided in two groups: a control group that consumed the non-enriched yogurt, and another group that consumed the protein-enriched yogurt. Each participant consumed a 100-g portion of the yogurt daily for 15 days. The yogurts were delivered weekly in packaging with the exact portion to be consumed at 4 °C. The participants were instructed to not alter their food habits and their practice of physical activities. At the end of the study, the control group was formed by 8 volunteers, whereas the protein-enriched yogurt group had 5 volunteers. On days 1 and 15, the volunteers were weighed using a weight scale with a 100-g graduation (model 31, Filizola, São Paulo, Brazil). The height of each volunteer was evaluated on day 1, using a stadiometer. Skinfold measurements were obtained with an adipometer (Cescorf, Brazil), with 10 g/mm^2 springs, and the body circumference was measured with an anthropometric tape (Cescorf, Porto Alegre, Brazil). The lean body weight and fat weight were evaluated according to the methods described by Jackson and Pollock (1978).

2.8. Electromyography and dynamometry

On days 1 and 15 of yogurt consumption, the volunteers underwent an electromyography, which was conducted with a Bagnoli 8-channel system (Delsys, Wellesley, MA, USA). Active electrodes were used for the pre-amplifier (gain: 1000), and band pass filtering was carried out at 20–450 Hz with a sampling frequency of 2000 (Hermens & Freriks, 1996). When necessary, trichotomy and asepsis with 70°GL alcohol were applied to diminish local impedance, and the electrodes were placed bilaterally according to the muscle fibers in the target muscles *(biceps brachii* and *quadriceps femoris*, both left and right), following the procedure proposed by the European project Surface EMG for Non-Invasive Assessments of Muscles Guide (SENIAM). The reference electrode was placed in the spinous process of the seventh cervical vertebra (Hermens & Freriks, 1996). A traction dynamometer was used to assess the strength of the elbow flexor and knee extensors according to a standard protocol. The random sequence order of direction (knee flexion and knee extension) and position (90°) was tested and established before starting the study. After a period of familiarization, the volunteers were warned to avoid explosive contractions and instructed to progressively increase their efforts up to the maximum strength level for each maximum static contraction. Verbal encouragement was provided during the maximum static contractions. The protocol involved three attempts for each position, with a minimum rest period of 30 s between each contraction. Each volunteer was given a 5-min rest period before repeating the entire procedure. Participants were instructed to abstain from caffeine, alcohol, and strenuous exercise for 36 h prior to each visit.

2.9. Statistical analysis

For the chemical analysis, syneresis analysis, microorganism enumeration, texture profile analysis, and sensorial acceptance test results, significant differences ($p < 0.05$) between the two yogurt formulations were evaluated with the Student *t*-test. Significant differences ($p < 0.05$) among the days of storage were evaluated with the Tukey test. The chi-square test was used to analyze differences in the consumer perceptions of the yogurts based on the CATA responses. The frequency of selection of each term for each sample was measured by counting the number of consumers that checked the term to describe each sample. Cochran's Q test was carried out on the CATA results to identify significant differences between the samples for each of the attributes. The Student *t*-test was also used to evaluate differences in the total body weight, lean body weight, and fat weight losses/gains ($p < 0.05$) as well as in the electromyography and dynamometry results. Statistica 13.0 software was used for all the analyses.

3. Results and Discussion

In the present study, whey protein concentrate supplementation was used to produce a protein-enriched yogurt. In the work of Oliveira et al. (2020), the authors used unripe banana and beet flours to produce an enriched yogurt with this protein compounds, demonstrating the nutritional and functional potential of the new product developed in their study.

The chemical compositions of the protein-enriched and non-enriched yogurts are presented in Table 1. As expected, the protein level in the enriched yogurt was significantly higher ($p < 0.05$) owing to the addition of the whey protein concentrate to the yogurt formulation. The contents of fats, total solids, and ash were also higher ($p < 0.05$) as a result of the protein addition. However, there was no difference in the lactose levels between the two formulations ($p > 0.05$). Jorgensen et al. (2019) attributed the "high-protein yogurt" label to products containing a minimum of 5.6% protein. Thus, the use of 15% skim milk powder in both formulations gives them the high-protein yogurt status. Moreover, the addition of 25% whey protein concentrate renders the enriched formulation a functional product, with higher levels of β-lactoglobulin, α-lactalbumin, branched-chain amino acids, and other active peptides also being formed after fermentation.

Table 1. Chemical composition and color of yogurt enriched with whey protein concentrate and of non-enriched yogurt.

Component	Enriched yogurt	Non-enriched yogurt
Protein (g 100 g^{-1})	13.66 ± 0.95^a	6.20 ± 0.45^b
Fat (g 100 g^{-1})	1.00 ± 0.00^a	0.5 ± 0.00^b
Ash (g 100 g^{-1})	1.27 ± 0.02^a	1.12 ± 0.02^b
Lactose (g 100 g^{-1})	4.01 ± 0.04	3.48 ± 0.97
L*	83.58 ± 2.32^b	85.92 ± 2.65^a
a*	-1.11 ± 0.95^a	-2.07 ± 0.93^b
b*	14.76 ± 3.94^a	12.08 ± 3.13^b

Values are presented as Mean ± Standard Deviation
[a,b] Different superscript letters in the same row represent significant differences by Student t test (p < 0.05). Source: Authors.

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Table 1 also shows the L*, a*, and b* values obtained according to the CIE color scale. Color plays an important role in food choice by consumers. The protein-enriched yogurt was darker and yellower, whereas the non-enriched yogurt was greener by comparison ($p < 0.05$). These colors could be attributed to the light-yellow color of the whey protein concentrate, the intensity of which is proportional to the amount of whey added. González-Martínez et al. (2002) also reported a yellowish color development in yogurt to which whey powder had been added.

Table 2 presents the data on the pH, titratable acidity, syneresis, and microbial counts of the protein-enriched and non-enriched yogurts. The protein-enriched yogurt had a higher pH ($p < 0.05$) during the 35 days of cold storage, indicating that addition of the whey protein had resulted in a buffering effect. Walstra and Jenness (1984) affirmed that increasing the level of non-fat solids in the mixture results in an increase in the titratable acidity of the milk owing to the buffering effect of the additional proteins, phosphates, citrates, lactates, and other miscellaneous milk constituents. The same difference was observed for the titratable acidity, with higher levels of lactic acid recorded in the enriched yogurt ($p < 0.05$) (Table 2). Jorgensen et al. (2015) reported that yogurts with the longest fermentation times (highest casein, calcium, and phosphorus contents) also had the highest content of lactic acid in the final products.

The protein-enriched yogurt did not demonstrate any syneresis during the 35 days of cold storage (Table 2), whereas the non-enriched yogurt presented an approximately 10% degree of syneresis. Syneresis is caused by serum release from the gel matrix, is considered a major technological defect in yogurts. Indeed, the addition of whey proteins with high water holding capacity (Mahomud et al., 2017) leads to a reduction in syneresis (Delikanli; Ozcan, 2014).

The yogurts were in accordance with Brazilian legislation (Brasil, 2001) regard the presence of thermotolerant coliforms, coagulase-positive staphylococci, *Salmonella* spp., and *Listeria monocytogenes*.

The *L. bulgaricus* and *S. thermophilus* counts were not statistically different ($p > 0.05$) between the two formulations studied (Table 2). It is important to highlight that according to Codex Alimentarius (WHO – FAO, 2011), the total amount of the microorganisms constituting the starter culture in yogurt must be at least 7 Log CFU g^{-1} up to the date of minimum durability. At 35 days of storage, *L. bulgaricus* remained at approximately 6 Log CFU g^{-1} and *S. thermophilus* at approximately 8 Log CFU g^{-1} in both formulations.

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Table 2. pH, titratable acidity (% lactic acid), syneresis, and *Lactobacillus bulgaricus* and *Streptococcus thermophillus* counts of yogurt enriched with whey protein isolate and of non-enriched yogurt during cold storage.

Parameter	Days of storage	Enriched yogurt	Non-enriched yogurt
pH	1	4.59 ± 0.01^{A,a}	4.41 ± 0.17^{B}
	7	4.52 ± 0.10^{A,a,b}	4.43 ± 0.05^{B}
	14	4.52 ± 0.10^{A,a,b}	4.45 ± 0.09^{B}
	21	4.52 ± 0.05^{A,b}	4.33 ± 0.05^{B}
	28	4.51 ± 0.07^{A,b}	4.35 ± 0.03^{B}
	35	4.49 ± 0.04^{Aa,b}	4.37 ± 0.02^{B}
Titratable acidity (% lactic acid)	1	1.29 ± 0.24^{A}	0.89 ± 0.14^{B,a,b}
	7	1.50 ± 0.11^{A}	0.90 ± 0.13^{B,b}
	14	1.68 ± 0.08^{A}	1.03 ± 0.13^{B,a}
	21	1.48 ± 0.38^{A}	0.98 ± 0.10^{B,a,b}
	28	1.37 ± 0.26^{A}	1.04 ± 0.09^{B,a,b}
	35	1.26 ± 0.04^{A}	1.05 ± 0.02^{B,a,b}
Syneresis (g 100 g^{-1})	1	0.00 ± 0.00^{B}	9.9 ± 3.01^{A}
	7	0.00 ± 0.00^{B}	11.0 ± 2.00^{A}
	14	0.00 ± 0.00^{B}	9.4 ± 1.59^{A}
	21	0.93 ± 1.46^{B}	10.1 ± 4.26^{A}
	28	0.00 ± 0.00^{B}	10.7 ± 6.11^{A}
	35	0.00 ± 0.00^{B}	9.1 ± 6.20^{A}
***L. bulgaricus* (Log CFU g^{-1})**	1	7.09 ± 0.27^{a}	7.19 ± 0.23
	7	6.91 ± 0.37^{a,b}	6.71 ± 0.14
	14	6.23 ± 0.42^{b,c}	6.49 ± 0.09
	21	6.14 ±0.12^{c,d}	6.34 ± 0.47
	28	5.70 ± 0.09^{c,d}	6.17 ± 0.50
	35	6.42 ± 0.51^{d}	6.43 ± 0.13
***S. thermophillus* (Log CFU g^{-1})**	1	9.13 ± 1.36	8.98 ± 2.00
	7	9.08 ± 0.16	8.97 ± 0.32
	14	8.68 ± 1.16	8.47 ± 0.98
	21	8.74 ± 0.22	8.85 ± 0.22
	28	8.65 ± 0.21	8.99 ± 0.18
	35	8.15 ± 0.56	8.75 ± 0.16

^{A,B} Different superscript capital letters in the same raw represent significant differences in yogurt formulations in the same day by Student t test ($p < 0.05$).
^{a,b} Different superscript lowercase letters in the same column represent significant differences in yogurt formulations along storage by Tukey test ($p < 0.05$).
Source: Authors.

Proteins are central to the formation of acidic milk gels such as yogurts and impact the textural properties of the dairy product (Jorgensen et al., 2019). In this study, the addition of 25% whey protein concentrate to the yogurt promoted differences in the textural properties, increasing its cohesiveness (Table 3). Szczesniak (1963) defined the mechanical characteristics of foods as being the most important textural parameters in determining the way the food handles and behaves in the mouth. These characteristics are the hardness (the force necessary to attain a given deformation), cohesiveness (the strength of the internal bonds making up the body of the product), viscosity (the rate of flow per unit force), elasticity (the rate at which a deformed material goes back to its undeformed condition after the deforming force is removed), and adhesiveness (the work necessary to overcome the attractive forces between the surface of the food and the surface of other materials with which the food comes in contact, such as the tongue, teeth, palate, etc.). No statistical difference was observed ($p > 0.05$) between the two formulations with regard to hardness. This is contrary to the results obtained by Guzmán-González et al. (1999), who reported that whey protein concentrate addition to yogurt promoted a decrease in the firmness of the product, as observed from its lower viscosity. Those authors formulated yogurts with a final protein content of 4.3 g 100 g^{-1}, using skim milk powder or skim milk powder + whey protein concentrate, among other combinations. However, similar to our study, Delikanli and Ozcan (2014) also observed no statistical difference in hardness between their control yogurt and whey protein concentrate-supplemented yogurt.

Our protein-enriched yogurt had significantly higher cohesiveness ($p < 0.05$), which was in agreement with the results observed by Delikanli and Ozcan (2014). During heat treatment, whey protein denaturation occurs, resulting in an increase in the number of charged groups on the amino acids, which leads to a stronger gel structure and integrity and consequently greater cohesiveness.

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Table 3. Texture profile analysis of yogurt enriched with whey protein isolate and of non-enriched yogurt during cold storage.

Texture parameter	Days of storage	Enriched yogurt	Non-enriched yogurt
Hardness (N)	1	2.72 ± 0.62	3.16 ± 0.14^{a,b}
	14	2.62± 0.56	2.69 ± 0.45^{b}
	21	2.71 ± 0.62	2.35 ± 0.19^{b}
	28	3.18 ± 0.23	3.55 ± 0.79^{a}
	35	3.33 ± 0.14	3.64 ± 0.95^{a}
Adhesiveness (mJ)	1	2.32 ± 2.03^{a,b}	1.86 ± 0.18^{a}
	14	3.80 ± 1.05^{A,a}	1.42 ± 0.22^{B,b}
	21	1.42 ± 1.51^{a,b,c}	1.23 ± 0.20^{b,c}
	28	1.18 ± 1.41^{b,c}	0.76 ± 0.15^{d}
	35	0.31 ± 0.25^{B,c}	0.97 ± 0.30^{A,c,d}
Cohesiveness	1	0.56 ± 0.04^{A,a}	0.44 ± 0.06^{B,a}
	14	0.49 ± 0.04^{A,b}	0.38 ± 0.02^{B,b}
	21	0.56 ± 0.04^{A,a}	0.43 ± 0.03^{B,a,b}
	28	0.51 ± 0.07^{A,a,b}	0.46 ± 0.04^{B,b}
	35	0.49 ± 0.06^{A,b}	0.43 ± 0.03^{B,a}
Gumminess (N)	1	2.08 ± 0.54^{B}	2.81 ± 0.10^{A,a}
	14	1.91 ± 0.54	2.09 ± 0.71^{a,b}
	21	2.15 ± 0.50	1.80 ± 0.05^{b}
	28	2.38 ± 0.57	2.57 ± 0.56^{a,b}
	35	2.07 ± 0.49	2.55 ± 0.74^{a,b}

^{A,B} Different superscript capital letters in the same raw represent significant differences in yogurt formulations in the same day by Student t test ($p < 0.05$).
^{a,b} Different superscript lowercase letters in the same column represent significant differences in yogurt formulations along storage by Tukey test ($p < 0.05$).
Source: Authors.

Table 4 presents the sensory scores, overall acceptance, and purchase intention for the yogurts. No significant age-related differences were observed with respect to acceptance of the samples by the panelists, 23.2% of whom were under 20 years old, 53.6% were 31–30 years old, and 23.2% were over 31 years old. Regarding gender, most panelists (59 females; 36 males) were women (62.1%), whose reported frequencies of yogurt consumption were as follows: daily, 11.6%; weekly, 30.5%; monthly, 29.5%; and almost never, 28.4%.

Table 4. Sensory analysis of yogurt enriched with whey protein isolate and of non-enriched yogurt.

Attribute	Enriched yogurt	Non enriched yogurt
Appearance	5.1^{A}	4.5^{B}
Texture	5.0	4.6
Flavor	4.8	4.9
Arome	6.3	6.0
Overall acceptance	5.2	5.0
Purchase intention	2.6	2.6

^{A,B} Different superscript capital letters in the same raw represent significant differences in yogurt formulations by Student t test ($p < 0.10$).
Source: Authors.

Approximately half of the panelists practiced some form of physical activity (52.6%), with 48% performing cardiorespiratory exercises (walking, football, running, volleyball, basketball, cycling, and martial arts), 42% performing resistance exercises (weight training and Pilates), and 10% practicing both cardiorespiratory and resistance exercises. Panelists who practiced physical activity gave a higher texture score ($p < 0.05$) to the protein-enriched yogurt (5.4) compared with those who did not perform any physical activity (4.5). Generally, individuals who practice physical activities tend to consume protein supplements (e.g., whey protein) at some point in time and are accustomed to the taste that whey protein imparts to the products.

The majority of the panelists did not use nutritive supplements regularly (95.8%) and had already tasted high-protein yogurts (64.2%). Panelists who had experienced high-protein yogurts gave lower flavor (5.6 *versus* 6.7; $p < 0.05$) and purchase intention scores (2.3 *versus* 2.8) to the protein-enriched yogurt than did the panelists who had never tasted this kind of product. Moreover, 58.9% of the panelists indicated that they preferred sweetened to unsweetened yogurt.

Overall, no statistical differences were observed between the protein-enriched and non-enriched yogurts in terms of the sensory, overall acceptance, and purchase intention scores. The sole difference was regarding the appearance, for which the enriched yogurt received an average rating of 5.1 *versus* 4.5 for the non-enriched yogurt ($p < 0.10$).

Figure 1 presents the word clouds of the CATA responses. Among the 13 sensory terms listed in the CATA questionnaire, 11 presented significant differences between the samples (all except for acid and artificial flavors), indicating that the consumers had perceived differences in the sensory characteristics of the yogurts despite that they rated both products with similar scores (Table 4). The enriched yogurt was mostly characterized by the presence of whey protein (50.5% of panelists), sweetener flavor (43.1%), aftertaste (38.0%), curdled texture (34.7%), yellow color (33.7%), and sweetness (32.6), whereas the non-enriched yogurt was characterized by the curdled texture (56.8%), curdled aspect (41.1%), yellow color (37.8%), aftertaste (33.7%), sweetener flavor (30.6%), and artificial flavor (8.4%). With regard to the non-sensory attributes of the enriched yogurt, 48.4% of the panelists chose "It is a healthy option," 37.8% chose "It is a nutritious food," and 25.3% chose "It is a good breakfast choice."

Figure 1. Word clouds obtained from the Check-All-That-Apply questionnaire responses on yogurt enriched with whey protein isolate (a) and non-enriched yogurt (b).

Source: Authors.

Table 5 presents the total body weight, lean body weight, and fat weight differences after 15 days of consumption of 100 g of enriched or non-enriched yogurt by 13 physically active individuals. On average, the body mass index of the participants was 24.2 kg m^{-2} before the study and 24.2 kg m^{-2} after 15 days of study. For all these individuals, weight variation was observed through their loss and gain of weight. After 15 days of protein-enriched yogurt consumption, the individuals had lost 420 g against the gain of 1370 g of lean body weight. By contrast, the individuals who consumed non-enriched yogurt had lost 420 g against the gain of 2610 g of lean body weight. Regarding fat weight, the participants who had consumed the enriched yogurt had no loss/gain or a gain of 50 g, whereas those who consumed non-enriched yogurt had no loss/gain or a loss of 3670 g. No significant difference in terms of total body weight, lean body weight, or fat weight before and after yogurt consumption was observed for both formulations. It was concluded that the daily consumption of 13.66 g of milk proteins for 15 days by physically active individuals did not affect their lean body weight gain.

Table 5. Total weight difference, lean body weight gain, and fat weight gain of 13 individuals who performed physical activities after the consumption of 100 g of yogurt daily for 15 days.

Weight difference (g)	**Enriched yogurt**		**Non-enriched yogurt**	
	Male	**Female**	**Male**	**Female**
Total weight	70 (0) -1500 to 1700	-350 (-350) -700 to 0	100 (0) -2000 to 2400	-180 (-450) -2300 to 2500
Lean body weight	320 (0) -420 to 1370	-320 (320) -600 to 40	1570 (2120) -420 to 2610	170 (-50) -1710 to 2490
Fat weight	0 (0) 0	-30 (30) 0 to 50	-960 (-130) -3670 to 0	-110 (-60) -330 to 0

Data are presented as Mean (Median) minimum value to maximum value.
Source: Authors.

Table 6 presents the surface electromyography and dynamometry data. Surface electromyography is used extensively in research and clinical applications to measure the electrical activity within skeletal muscles. According to our results, there was no significant difference in electromyography responses after daily consumption of the enriched or non-enriched yogurt for 15 days ($p > 0.05$). The same was observed for the test of muscular strength ($p > 0.05$). However, there was a significant correlation ($p < 0.05$) between both measures, where physically active individuals who had consumed the protein-enriched yogurt exerted major muscle strength with less muscle fiber activation.

The study made it possible to evaluate the development of a protein-enriched yogurt. It was also possible to evaluate the effect of yogurt on the gain of lean body weight gain and electrical activity in skeletal muscle of physically active individuals. The main limitation of the study was the small number of individuals evaluated, since most of those approached scheduled anamnesis and did not attend.

Table 6. Electromyography and dynamometry results for 13 individuals who performed physical activities before and after consumption of 100 g of protein-enriched yogurt or non-enriched yogurt daily for 15 days.

Target muscle	Enriched yogurt				Non-enriched yogurt				p[†]
	Before	**After**	**Δ**	**p**	**Before**	**After**	**Δ**	**p**	
Electromyography (Hz)									
Right FR	29.06 ± 9.79	27.31 ± 8.16	1.75	0.60	32.65 ± 12.04	30.71 ± 9.39	1.94	0.54	0.25
Left FR	30.48 ± 12.37	30.18 ± 12.99	0.30	0.94	33.23 ± 12.71	30.74 ± 10.44	2.49	0.46	0.88
Right BB	21.02 ± 10.19	19.26 ± 6.32	1.76	0.57	23.07 ± 8.50	25.38 ± 12.17	-2.31	0.45	0.08
Left BB	21.83 ± 10.10	19.92 ± 6.87	1.91	0.55	22.41 ± 8.22	24.71 ± 12.05	-2.30	0.45	0.17
Dynamometer – Muscular Strength (Kg)									
Right FR	114.17 ± 42.04	114.55 ± 69.58	-0.38	0.99	118.27 ± 84.78	180.73 ± 242.12	-62.46	0.24	0.31
Left FR	124.99 ± 62.20	121.85 ± 62.57	3.14	0.89	101.96 ± 47.90	136.98 ± 89.79	-35.02	0.10	0.57
Right BB	541.97 ± 324.31	487.68 ± 219.18	-42.71	0.60	513.34 ± 287.89	483.13 ± 354.01	30.21	0.75	0.96
Left BB	469.71 ± 188.41	556.14 ± 264.83	-86.43	0.31	496.60 ± 359.88	480.17 ± 333.58	16.43	0.87	0.46

FR= *Femoral Rectum*; BB= *Biceps brachial*.
Δ= Difference between values obtained before and after yogurt's consumption.
[†] Indicate statistical significance between enriched and non-enriched yogurt results after yogurt consumption.
Source: Authors.

4. Final Considerations

The addition of whey protein concentrate to the yogurt formulation conveyed a functional characteristic to the dairy product. Despite its greater amount of protein, the textural parameters of the enriched yogurt were generally not altered. Although the sensory analysis indicated no differences between the protein-enriched and non-enriched yogurts, the descriptive analysis revealed that the panelists perceived differences in the sensory characteristics. Consumption of the protein-enriched yogurt did not influence the lean body weight gain by physically active individuals.

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