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ANALYSIS OF CARBONYLATED PROTEINS IN CAKE FORMULATIONS ADDED WITH WHEY PROTEIN

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ABSTRACT

Chemical modifications in cake formulations made with whey protein and subjected to high temperatures undergo the formation of carbonylated proteins which is a biomarker of protein oxidation. Cake formulations were prepared with and without the addition of whey protein, divided between raw and baked, amounting to a total of 4 samples. The chemical composition was analyzed in triplicate, following methodologies described by AOAC. The carbonylated proteins were quantified by spectrophotometry in the Food Analysis Laboratory of the Department of Nutrition at the Federal University of Triangulo Mineiro. The results were presented as mean ± standard deviation and analyzed by ANOVA and Tukey's post-test, adopting as significant at $p<0.05$. Raw samples showed higher water content compared to roasted, and a higher amount of ash in the sample added with whey protein. The amount of protein was higher in samples with whey protein. The sample baked with whey protein had a higher amount of carbonylated proteins in relation to others. The addition of whey protein positively influenced the increased formation of carbonylated proteins in the cake. The intake of oxidized proteins can lead to disorders and diseases and should be avoided.

Key words: Carbonylated proteins. Maillard reaction products. Whey protein.

RESUMO

Análise de proteínas carboniladas em formulações de bolos adicionadas com whey protein

Modificações químicas em formulações de bolos elaborados com whey protein e submetidos a altas temperaturas sofrem a formação de proteínas carboniladas que é um biomarcador de oxidação proteica. Foram elaboradas formulações de bolo com e sem adição de whey protein, divididos entre cru e assado, totalizando 4 amostras. A composição química foi analisada em triplicata, seguindo metodologias descritas pela AOAC. As proteínas carboniladas foram quantificadas por espectrofotometria no Laboratório de Análise de Alimentos do Departamento de Nutrição da Universidade Federal do Triângulo Mineiro. Os resultados foram apresentados como média ± desvio padrão e analisados por ANOVA e pós-teste de Tukey, adotando-se como significativo $p<0,05$. As amostras cruas apresentaram maior teor de água em relação às torradas, e maior quantidade de cinzas na amostra adicionada de whey protein. A quantidade de proteína foi maior nas amostras com whey protein. A amostra assada com whey protein apresentou maior quantidade de proteínas carboniladas em relação às demais. A adição de whey protein influenciou positivamente no aumento da formação de proteínas carboniladas no bolo. A ingestão de proteínas oxidadas pode causar distúrbios e doenças e deve ser evitada.

Palavras-chave: Proteínas carboniladas. Produtos da reação de Maillard. Proteína de soro.

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INTRODUCTION

Milk proteins may be classified into two major groups: casein is about 80% of milk protein and whey proteins that account for the remaining 20%. The properties of whey protein began to be studied by scientists since the 70's until whey protein was discharged by the food industry (Lonnerdal, 2003).

In recent decades, many researchers have shown the nutritional qualities of whey protein (Chevalier et al., 2001; Foegeding et al., 2002; Haraguchi, Abreu, Paula, 2006).

Whey proteins are extracted from the aqueous portion of milk during the cheese manufacturing process and transformed into powder by lyophilization or spray drying. Therefore, the composition of the powder of whey protein depends on the type, culture, and cheese processing conditions (Hurley et al., 1990).

Variations in dairy cows and sheeps body composition may also affect protein levels (Mehra et al., 1999).

Whey protein has been widely used by the food industry as a functional ingredient in foods (Chevalier et al., 2001; Foegeding et al., 2002).

Practitioners of physical activities have sought benefits from this protein source since nutrition has an important role in this group and sedentary individuals require less protein amount stipulated for physically active people and athletes (Lemon, 1998).

Thus, the protein recommendation is 1.2 to 1.6 g per kilogram body weight per day for people practicing endurance training, 1.6 to 1.7g / kg/day for strength athletes, approximately twice the current IRD, which is 0.8-1.0g / kg/day established for sedentary people (Lemon, 1998).

Whey protein has a high concentration of essential amino acids (AA). These include cysteine, lysine, tryptophan, and branched-chain amino acids (BCAA) (leucine, isoleucine, and valine), which promote anabolism as well as a reduction of protein catabolism, favoring the gain of muscular strength and reducing the loss of muscle mass during weight loss.

The intake of protein or AA after exercise benefits recovery and muscle protein synthesis (Lemon, 1998; Sgarbieri, 2004).

Thus, the use of supplements with whey protein has increased among athletes and practitioners of physical activity, aiming to increase the biological value of dietary proteins and promote their anabolic effects.

Due to the periodic and monotonous intake of whey supplements, which can be tedious, this group has been looking for new ways to be able to continue using these supplements.

Thus, crude formulations such as in addition to fruit juices or with thermal processing such as with cakes and cookies have been used.

Before being consumed most food undergoes thermal processing, guaranteeing their microbiological safety, the deterioration of toxic and antinutritional factors, inactivating some enzymes, and also the development of substances responsible for the aroma, color, and flavor improving the palatability (Oliver, Melton, StanleY, 2006).

In milk and dairy products, the Maillard reaction (MR) from heat treatments applied in processing technology is a common development.

The MR gives valuable sensory attributes for thermally processed foods due to the generation of volatile compounds (Grossin et al., 2015) responsible for flavor and taste (aldehydes and ketones), color (melanoidins), and texture. Moreover, some compounds from MR may cause adverse effects on human health, such as acrolein and aromatic heterocyclic amines (Barbosa, Oliveira, Seara, 2009).

The classical pathway of the MR or glycation begins with the formation of an unstable Schiff base generated by condensation of the carbonyl group of a reducing sugar with amine groups, for example, from the lateral chain of AA residues. Then, this base undergoes rearrangement, forming the Amadori product, known as the initial product of the MR.

These initial products have reactive carbonyl groups, which condense with accessible primary amine groups, giving rise to advanced products of the MR or advanced glycation end-products (AGEs).

The most widely used marker to indicate protein modifications is the content of carbonyl groups. The carbonyl groups are produced in protein side chains when oxidized as a result of reactions with AGEs. These portions are chemically stable, and useful for detection (Dalle-Donne et al., 2003).

The diet is currently considered the most important exogenous source of AGEs

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(Dalle-Donne et al., 2005) which are absorbed by the intestine and are the major contributors to the pool of AGEs in the organism (Uribarri et al., 2005).

The accumulation of carbonylated proteins can be observed in various diseases including Alzheimer's disease, diabetes, inflammatory bowel disease (IBD), and arthritis, among other pathologies associated with AGEs which occur due to their capacity to modify the chemical properties and functions of biological structures (Dalle-Donne et al., 2003; Liu et al., 2016).

This accumulation of carbonylated proteins promotes oxidative stress, morphological changes, and increased expression of inflammatory mediators from the generation of free radicals, crosslinking with proteins, or interactions with cellular receptors (Barbosa, Oliveira, Seara, 2009; Chevion, Berenshtein, Stadtman, 2000; Hayase et al., 1996).

Moreover, even in healthy individuals the blood concentration of AGEs is correlated with the increase in risk factors for diabetes and cardiovascular diseases (Poulsen et al., 2013).

Thus, this study aimed to quantify the chemical changes when recipes with whey protein are subjected to high temperatures, since there may be a formation of carbonylated proteins.

MATERIALS AND METHODS

Two formulations of cakes were prepared, the first without the addition of whey protein and the second with the addition of whey protein.

The ingredients used and their amounts are listed in Table 1.

Table 1 - Ingredients used for formulations.

	Without whey protein	Added whey protein
Whole-wheat flour (g)	100	100
Brown sugar (g)	100	100
Egg (unit)	2	2
Natural yogurt (g)	100	100
Soy oil (mL)	8	8
Cocoa powder (g)	15	15
Baking powder (g)	3	3
Whey protein (g)	0	30

For each formulation, half was separated for analysis in a raw state and the other half was baked totaling 4 different samples: Raw without whey protein (RWWP), Raw with added whey protein (RAWP), Baked without whey protein (BWWP), Baked with added whey protein (BAWP).

Chemical analyses were performed in triplicate in the Food Analysis Laboratory of the Department of Nutrition at the Federal University of Triangulo Mineiro. Moisture, crude protein (N x 6.25), and ash contents were determined according to the Association of Official Analytical Chemists (1990) methods (Association of Official Analytical, 1990).

Total lipids were extracted and quantified following the method of Bligh and Dyer (1959) (Bligh, DyeR, 1959). Carbohydrate content was determined by calculating the difference between the other components.

The determination of carbonylated protein was performed by a reaction with dinitrophenylhydrazine (DNPH) to form hydrazones and quantified by spectrophotometry. Briefly, 0.5 g samples were homogenized with a Potter in 5 ml of distilled water and then diluted to 10 ml in a volumetric flask. The proteins were precipitated with an equal volume of 20% TCA, and the supernatant was discarded.

The precipitates were resuspended in 1 ml of 10 mM DNPH in HCl 2N and allowed to react for 1 hour at room temperature protected from light while stirring in a vortex every 15 minutes.

Afterward, the same volume of 20% TCA was added and centrifuged at 3000 rpm for 10 minutes at 4 ° C. The supernatant was discarded and the resulting precipitate was washed 3 times with ethanol/ethyl acetate (1: 1, v: v) repeating the centrifugation process to remove traces of DNPH and lipids. Then, the washed precipitate was resuspended in 1 mL of 6M guanidine and left in a water bath at 37 ° C for 1 hour, vortexing occasionally. New centrifugation was performed for 15 minutes at

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3000 rpm and the supernatant was read in a spectrophotometer at 370 nm to determine the content of carbonylated proteins. The calculations were made using a molar extinction coefficient of 22000 L / (mol. cm).

Results are presented as mean ± standard deviation. Differences of means were tested by the Analysis of Variance - ANOVA and the post hoc of Tukey. A P-value of <0.05 was considered statistically significant for all analyses.

RESULTS

In Table 2 are shown the chemical composition of the formulations in this study. The moisture of raw samples (RWWP and RAWP) were higher than baked (BWWP and BAWP) and all samples were different from one another. Inversely, the crude ash content was higher for BWWP and BAWP compared with RWWP and RAWP and the samples with whey protein were higher than the samples without whey protein.

As expected, the amount of protein was higher ($p<0.05$) for the formulations with added whey (15.2±1.2 g/100g and 14.0±1.5 g/100g for RAWP and BAWP, respectively) compared with the formulations without whey (8.7±0.3 g/100g and 9.2±0.3 g/100g for RWWP and BWWP, respectively). The total lipid content and carbohydrate by difference had its lowest value in RWWP.

Figure 1 shows the carbonylated protein values of the samples. It was found that the value of the carbonylated protein found in the AW sample (648.3 ± 88.2 µmol / 100g) was significantly ($p<0.05$) higher when compared to the values of CSW (417.5 ± 11.4 µmol / 100g), CW (357.2 ± 12.5 µmol / 100g) and ASW (463.1 ± 15.2 µmol / 100g).

DISCUSSION

The knowledge about chemical modifications from a nutritional point of view is important when concerning whey protein recipes, since when they are associated with high temperatures they undergo protein oxidation, in addition to changing the product's centesimal composition.

Table 2 - Chemical composition (mean ± standard deviation), on wet basis, of different formulations (g/100g).

	RWWP	RAWP	BWWP	BAWP
Moisture	39.0±0.1 a	37.4±0.1 b	32.1±0.1 c	29.9±0.2 d
Crude ash	0.9±0.0 a	1.0±0.0 b	1.1±0.0 c	1.2±0.0 d
Protein (N x 6.25)	8.7±0.3 a	15.2±1.2 b	9.2±0.3 a	14±1.5 b
Lipids	5.8±0.2 a	3.7±0.2 b	5.2±0.1 a,c	5.0±0.3 c
Carbohydrate	54.7±0.4 a	42.8±1.1 b	52.4±0.5 a,c	49.8±1.3 d

RWWP - Raw without whey protein, RAWP - Raw with added whey protein, BWWP - Baked without whey protein, BAWP - Baked with added whey protein. Different letters in same lines mean $p<0.05$.

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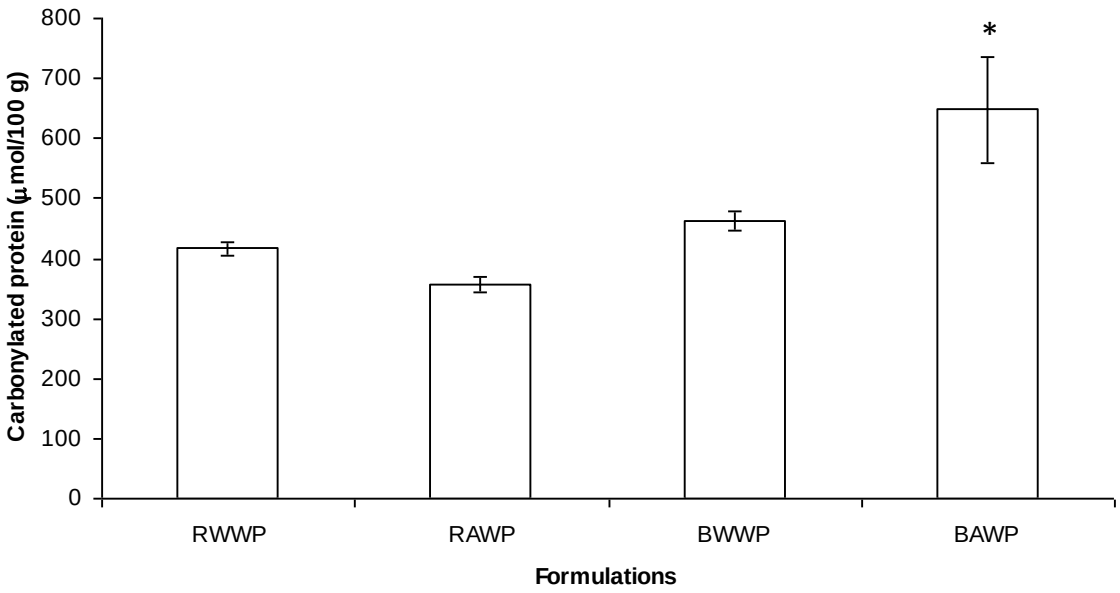

Figure 1 - Comparison of the content of carbonylated protein in the different samples. RWWP - Raw without whey protein, RAWP - Raw with added whey protein, BWWP - Baked without whey protein, BAWP - Baked with added whey protein. * p<0,05.

None unexpected changes occurred in our macronutrient comparisons. However, there was a significant increase in the carbonylation content of the proteins when the added whey formulation was subjected to heating.

This is particularly worrisome due to many young practitioners of physical activity engaging in permanent ingestion of preparations that undergo heating to break the routine of supplementing whey protein.

Although an acute intake appears to produce no toxic effects (Liu et al., 2016), studies with mice have shown that chronic ingestion even at low concentrations causes liver, kidney, endothelial and spinal damage (Grossin et al., 2015; Illien-Junger et al., 2015; Liu et al., 2016).

CONCLUSION

The present study demonstrates that a simple routine practice such as adding ingredients for supplementation could be harmful to health.

These data should be disseminated to professionals involved in nutritional counseling as well as to the public. In addition, new research on the subject should be encouraged to increase the body of evidence.

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