Structural Observations of Beef Burger containing Wheat Fiber of Different Sizes

Observações Estruturais de Hambúrguer Adicionado de Fibra de Trigo com Diferentes Granulometrias

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Abstract

The objective of the present study was to develop different formulations of functional beefburgers with addition of wheat fiber of different granule sizes, and to evaluate the effect of fibers on the structural and physical properties of the product. The fibers used were Fiber 200 (250 µm in length and 25 µm in thickness) and Fiber 600/30 (35 µm in length and 20 µm in thickness), and the addition followed a 2^2 central composite design. The fiber addition altered the physical and structural properties of the burgers. Significant differences were observed ($p < 0.05$) in the physical properties of the burgers due to both the variations in particle size of the wheat fiber, and the different concentrations. Comparing the observations found through microscopy with the results of shear force and cooking losses, it was concluded that there was a relationship between some properties and their respective microscopic structures. The best results were found for the treatment F2, indicating that the Fiber 200 (larger particles) allowed the maintenance of a texture close to the standard, F1 (without fiber addition), and lower cooking loss when compared to the product containing fiber of lower particle size (Fiber 600/30). The fiber mixtures in the proportions studied were not a viable alternative due to the increase in the hardness of the product.

Keywords: Microscopy. Functional Food. Dietary Fiber.

Resumo

Objetivou-se com o presente trabalho desenvolver diferentes formulações de hambúrguer funcional de carne bovina, com a adição de fibra de trigo com diferentes granulometrias e avaliar o efeito das fibras nas propriedades físicas e estruturais do produto. As fibras testadas foram denominadas de Fibra 200 (250 µm de comprimento e 25 µm de espessura) e Fibra 600/30 (35 µm de comprimento e 20 µm de espessura) e a adição seguiu um Delineamento Composto Central 2². A adição das fibras alterou as propriedades físicas e estruturais dos hambúrgueres desenvolvidos. Foram observadas diferenças significativas (p <0,05) nas propriedades físicas dos hambúrgueres, decorrentes da variação da granulometria da fibra de trigo e das concentrações testadas. Comparando as observações encontradas na microscopia com os resultados das avaliações de força de cisalhamento e perdas por cocção, conclui-se que existe relação entre algumas propriedades com as respectivas estruturas microscópicas. Os melhores resultados foram encontrados para F2, indicando que a Fibra 200 (maior granulometria) permitiu a manutenção da textura próxima do padrão F1 (sem fibra) e menores perdas por cocção, quando comparado com o produto adicionado da fibra de menor granulometria (Fibra 600/30). A mistura das fibras nas proporções testadas não se mostrou uma alternativa viável devido o aumento na dureza do produto.

Palavras-chave: *Microscopia. Alimento Funcional. Fibras na Dieta.*

1 Introduction

The increased demand for functional products offers great opportunities for the meat industry to search strategies for the development of these products, including addition of micronutrients to the formulations, and elimination of undesirable components, which can be achieved from changes in animal feeding or by reformulating meat products[1-3]. However, it is important to understand the technological aspects and the best way to apply these functional ingredients to meat products without losing their physicochemical and sensory characteristics.

Dietary fiber consists of substances whose source can be either animal or plant and which are resistant to hydrolysis of enzymes in the gastrointestinal tract[4]. The use of dietary fiber in food is of great interest in the area of health, once several studies have reported the role of dietary fiber in the prevention of diseases such as colon cancer, obesity, diverticulitis, cardiovascular problems, and diabetes[5-9]. Moreover, dietary fibers are desirable, not only for their nutritional properties, but also for presenting functional, technological and economic properties[10] because they possess the ability to form gels, retain water and fat, and increase viscosity, which may influence the texture, formation and stability of emulsion[11]. There have been studies regarding the use of orange, sugar beet, and wheat fiber in emulsified meat products such as sausages and mortadelas[7,12,13]. However, there are few studies on restructured products such as beefburgers[14,15], and there is also little research assessing the influence and functionality of wheat fiber of different particle sizes in the physical and structural characteristics of the final product.

The objective of this study was to develop different formulations of functional beefburger with the addition of wheat fiber of different particle sizes, and to evaluate the effect of the fiber on the physical and structural properties of the product using light microscopy technique.

2 Material and Methods

The experiments were performed in the laboratories of the Department of Food Science and Technology, Federal University of Santa Maria (UFSM). The project was approved by the Ethics Committee of UFSM (CAAE: 07188612.6.0000.5346).

Microscopy was performed in the histology laboratory of the Department of Pharmacy, of the Integrated Regional University of Alto Uruguai e das Missões (URI). The manufacture of the burger formulations followed the Technical Regulation of Hamburger Identity and Quality[16], Ordinance No.1004[17], and a 2^2 central composite design was used. The raw material consisted of beef donated by the Central Western Cooperative of Santa Catarina (Aurora Alimentos). The meat was minced (disc 5 mm) and sent to a mixer where the ingredients and additives were added. The base formulation consisted of: beef (84.00%), soy protein (4.000%), water (3.000%), hamburger seasoning (1.500%), garlic powder (1.200%), sodium chloride (0.700%) tripolyphosphate glutamate (0.500%), sodium (0.300%), maltodextrin (0.300%), parsley (0.200%), erythorbate (0.100%), smoke (0.040%), sodium lactate (0.010%), nitrite (0.010%) and carmine dye (0.002). The other ingredients, which differed in % according to the experimental design, are shown in Table 1. Three replicates were performed for each test.

Table 1: Central composite design (CCD) 2^2 for the development of hamburgers containing wheat fiber (real and coded values).

Ingredients*	Fiber 200 (%)	Fiber 600/30 (%)
T1	0.0	0.0
	(-1)	(-1)
T2	4.0	0.0
	(+1)	(-1)
T3	0.0	4.0
	(-1)	(+1)
T4	4.0	4.0
	(+1)	(+1)
T5**	2.0	2.0
	(0)	(0)

* % refers to the percentage of ingredients added in the final product.
** refers to the Central Point (CP).

The fibers were donated by the Rettenmaier Latinoamericana company (São Paulo, SP) and Fiber 200 (250 μm in length and 25 μm in thickness) and Fiber 600/30 (35 μm in length and 20 μm in thickness), both by Vitacel®, and consisting of 74% cellulose, 26% hemicelluloses, and 0.5% lignin. For all formulations, the fibers were added at the end of the manufacturing process.

After the preparation of the mixture and manual molding, the samples were packed and kept in a freezer (-18 °C) in polyethylene packaging, prior to analysis. The following determinations were performed: cooking losses and shear. The losses were assessed according to the methodology described by Ramos e Gomide[18] with some adaptations. The frozen burgers were wrapped in aluminum foil and taken to the oven at 260 °C, until the internal temperature of the burgers was up to 72 °C. The cooking loss was calculated as a percentage difference on weight loss. After cooking and cooling, the samples were cut (3 cm wide, 1 cm thick and 2.5 cm in length) and the shear strength was measured with the aid of TA-XT.plus texture analyzer, and the results were analyzed using specific software (Stable Microsystems Ltd., Surrey, England).

For light microscopy, three fragments were removed from each treatment, corresponding to the center of the hamburger. The material was processed according to the conventional technique cited by Junqueira and Carneiro[19]. The samples were qualitatively evaluated for the presence of collagen and muscle fibers, fat, fibrous and compact tissue, edema, cytoplasmic vacuoles, disruption, disorganization and binding tissue. Three slides were prepared for each treatment. The samples were cut into sections with a thickness of 4 mm and then stained with hematoxylin and eosin. A Leica microscope (Leica Microscopy Systems, Heerbrugg, Switzerland) was used, and Motic Images Plus 2.0. (Motic Instruments, Inc., Richmond, Canada) software was used for capturing images.

Three replicates were performed for each test. The results were submitted to analysis of variance - ANOVA, and Tukey's test at a significance level of 95% ($p < 0.05$). All results were analyzed using Statistica® 9.0 software (STATSOFT, INC).

3 Results and Discussion

There was significant difference ($p < 0.05$) for shear force and cooking losses among the different formulations (Table 2). The treatment F3 (4% Fiber 600/30) had the highest softness (lowest shear force) for the higher water absorption due to the smaller particle size and larger surface area of the wheat fiber, allowing a greater water absorption. The treatments F4 and F5 showed the highest shear values, indicating that the fiber mixture in a greater and lesser proportion has caused an increase in hardness when compared to both the product without fiber, and those containing fiber alone.

Table 2: Shear force and cooking losses for the different beefburger formulations containing wheat fiber of different particle sizes.

Treatments*	Shear (Kgf)	Cooking Loss (%)
F1	$1.65^b \pm 0.44$	$13.55^c \pm 1.05$
F2	$1.60^b \pm 0.59$	$10.07^b \pm 0.90$
F3	$1.06^a \pm 0.26$	$11.80^{bc} \pm 0.60$
F4	$1.91^c \pm 0.39$	$6.59^a \pm 0.50$
F5	$1.85^c \pm 0.32$	$6.72^a \pm 0.38$

* Means (± SD), n = 15. ± Standard Deviation with different letters vertically differ significantly (p < 0.05).
**F1: 0% fiber, F2: 4% Fiber 200, F3: 4% Fiber 600/30, F4: 4% Fiber 200 + 4% Fiber 600/30, and F5: 2% Fiber 200 + 2% Fiber 600/30. n = 3.

Choe *et al*.[20] reported an increase in hardness of sausages containing a mixture of pork skin and wheat fiber in the ratios of 10, 15 or 20%. Choi *et al*.[10] observed a decrease in hardness of gels formulated with rice bran fiber at concentrations above 2%. Choe *et al.*[11] observed an increase in hardness of sausages with the addition of 10, 15 and 20% pig skin and wheat fiber mixture. Sánchez-Alonso *et al*.[15] also observed an increase in the shear force of restructured fish product from 5.85 N/g (control) to 7.26 N/g with the addition of long fiber (larger particle size) (3%), and 6.14 N/g with the addition of short fiber (smaller particle size).

In contrast, Cordeiro *et al*.[14] found a reduction in the shear force of beefburger with wheat fiber (0%, 2% and 4%), indicating a positive effect of fiber on the softness of the product. Jimenez-Colmenero *et al*.[20] also found that the hardness of sausages was reduced from 20.78 N (control) to 14.13 N with the fiber addition.

A possible explanation for the increased toughness with the addition of fiber with larger granule size (Fiber 200) when compared to both the fiber with smaller particle size (Fiber 600/30) and the fiber mixture is that, according to Sánchez-Alonso *et al.*[15] , the fiber probably absorbed water and the muscle particles became harder.

In the present study, the treatments with the lowest cooking losses were F4 and F5 (Table 2), indicating that higher percentages of fiber and a mixture of these fibers provided greater resistance to water and fat loss during the cooking process. The treatment F1 (0% fiber) had the highest cooking loss. This result is interesting from the technological aspect, because a product with lower cooking loss will probably remain juicier and more enjoyable for consumers. None of the formulations fragmented during cooking, which is not in accordance with the study by Cordeiro *et al.*[14] where the addition of 6% fiber caused this problem.

Choe *et al.*[11] observed a reduction in cooking loss from 6.53 ± 0.58 (control) to 4.76 ± 0.58 by adding 10% of a mixture of wheat fiber and pig skin, and Jimenez- Colmenero *et al*.[20] reported a loss of 8.9% in sausages with 20% WF200 wheat fiber (the same used in this study). Yang *et al.*[12] also observed reduced losses during cooking with the addition of hydrated oatmeal and tofu in sausages.

Sánchez-Alonso *et al*.[15] found a reduction in cooking loss in restructured fish products with the addition of long wheat fiber (3 or 6%), and short fiber (3%), but the losses were higher when using the long fiber (large particles). There were significant differences (p <0.05), mainly when a fiber mixture was used, in comparison with the use of a single fiber.

Losses during cooking are important because they affect the appearance and juiciness of the product. The results showed that the fiber mixture improved the performance and efficiency of these ingredients in the meat product, resulting in reduced cooking losses.

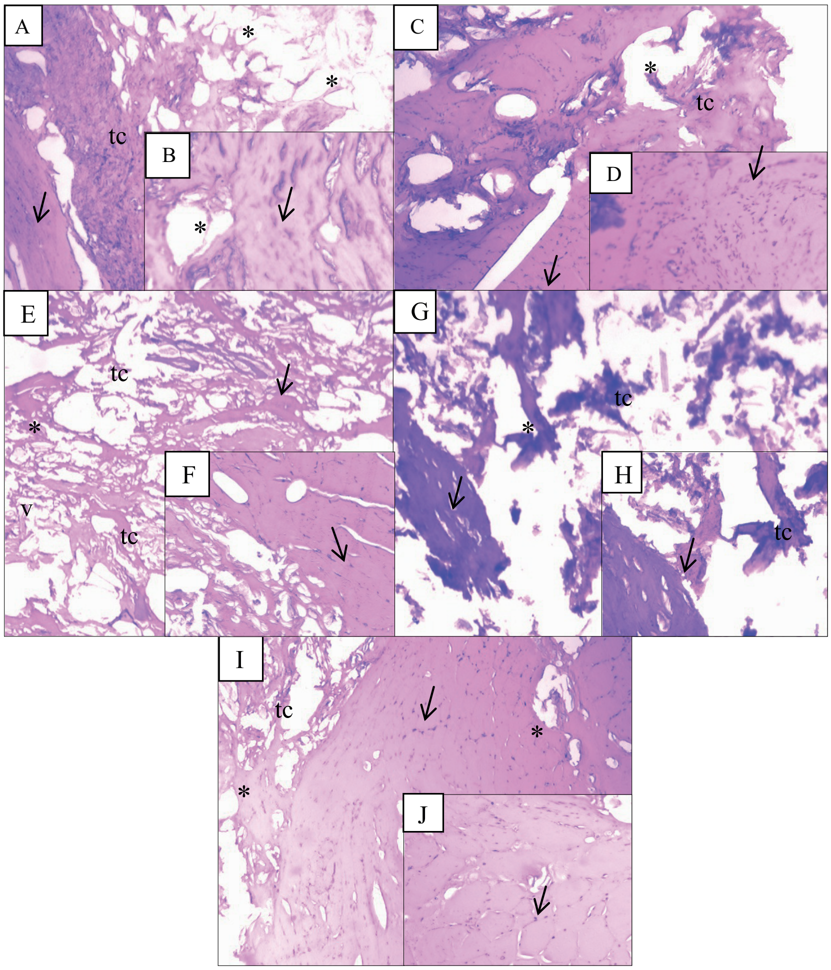
Table 3 shows the effects of the input variables (% Fiber 200, Fiber 600/30 and their mixtures) in the variable cooking loss (R^2 = 69.38%). It can be seen that only the first order effects were significant (p <0.05). Both variables showed a negative effect on cooking losses, which is favorable for the product because it indicates loss reduction. The effects for the fiber mixture (second order) were not significant (p> 0.05). It was noted that the use of Fiber 200 (larger particle size) alone provided lower cooking loss when compared to the Fiber 600/30 (smaller particle size), which was desired and may represent a technological advantage.

Table 3: Percentage effects of the input variables for Fiber 200, Fiber 600/30 and their mixtures in the variable cooking loss (%) for the formulations of beefburger.

	Cooking losses (%)		
	Effects	Standard Deviation	p
Average/Interaction	9.406	0.164	<0.001*
(1) Fiber 200	-4.283	0.367	<0.001*
(2) Fiber 600/30	-2.402	0.367	<0.001*
1X2	-0.883	0.367	0.061

* Significance of 95%.

Figure 1 shows the photomicrographs (A to J) obtained by the hematoxylin and eosin method for the different treatments. Microscopy was used to evaluate the relationship between the physical properties of the food system with their respective microscopic structures. Through microscopy we were able to observe the collagen and muscle fibers, fat, fibrous and compact tissue, edema, cytoplasmic vacuoles, disruption, disorganization and binding tissue.



Detail A, B) corresponds to F1, (*) and the fibrous, fairly compact, adjacent connective tissue (tc). Presence of skeletal muscle fibers (arrows). C, D) F2. Muscle fibers with peripheral nuclei, and proliferation (arrows) as well as the reduction of fibrous tissue (tc) and the reduction of interstitial edema (*). E, F) T3 showing intense disruption of the collagen fibers (tc). Note the cytoplasmic vacuoles (v). G, H) T4. Note the disintegration tissue (tc) shown by the lack of sharpness (arrows) and cytoplasmic vacuoles (v). Note the disorganization of collagen fibers (tc). I, J) T5 showing the organization of collagen fibers (tc) and the layout of the muscle fibers of normal appearance (arrows). Presence of edema a lower intensity (*). 4x and 10x, respectively. Hematoxylin and eosin.
* F1: 0% fiber F2: 4% Fiber 200, F3: 4% Fiber 600/30, F4: 4% Fiber 200 + 4% Fiber 600/30, and F5: 2% Fiber 200 + 2% Fiber 600/30.

Figure 1: Photomicrographs of the different beef burger formulations.

It was found that the addition of wheat fiber affected the structure of the product. The photomicrographs A and B corresponded to treatment F1 (without fiber addition), while C and D correspond to the addition of larger granule fiber (Fiber 200), and E and F correspond to the addition of smaller granule fiber (Fiber 600/30). The photomicrographs G and H, and I and J corresponded to the treatments containing the fiber mixture (4% Fiber 200 and 4% Fiber 600/30, and 2% Fiber 200 and 2% fiber 600/30, respectively). The photomicrographs C and D, and I and J show a more cohesive and compact matrix structure when compared with photomicrographs E and F, and G and H, probably due to the regular distribution of the fiber with larger particles (Fiber 200) that occupied spaces in the protein matrix and made the network more homogeneous.

The photomicrographs C and D correspond to the treatment F2, and it can be seen that the more compact and cohesive structure retained more moisture, presenting softness similar to the treatment F1 (without fiber addition), and the cooking losses were less than the treatment F3 (more disorganized structure). In addition, it is noteworthy the reduction of edema due to the incorporation of water in relation to the treatment F1 (micrographs C and D, as compared to A and B).

Photomicrographs E and F correspond to the treatment F3, in which only Fiber 600/30 (smaller particle size) was added.

The structure was very close to the treatment F1 (without fiber addition), which can probably be due to the smaller fiber size which resulted in a higher, but not homogeneous, distribution and did not result in a more cohesive and compact matrix. The effect of larger particle-sized fiber can also be explained due to the coarse particle size of the meat used as a raw material for the burgers (5 mm), which may have affected the performance of the smaller particle-size fiber when compared to the larger-sized fiber. The degree of comminution or grinding can affect the interactions, and the effects can vary.

Photomicrographs G and H correspond to the treatment T4, and it can be seen that there was an intense structural disorganization when the maximum levels of fiber mixture (4% Fiber 200 and 4% Fiber 600/30) were added. The treatment T5 (2% Fiber 200 and 2% Fiber 600/30) showed a structure similar to the treatment T2.

Bortoluzzi[21] evaluated samples of chicken mortadela containing 1, 2, or 3% orange fiber and found higher emulsion stability and entrapment of fat globules resulting from the fiber addition.

Sachez-Alonso *et al*.[15] used scanning electron microscopy to evaluate surimi with wheat fiber of different sizes. The fiber affected the structure of the gel matrix. The distribution of the fiber in the matrix was shown to be regular and occupied spaces in the matrix. However, when the fiber size was larger (larger particle size) than the cells of the matrix, the network became less heterogeneous. Changes in the gel texture were observed, and the greater fiber distribution decreased the compression of the gel structure.

The treatment F3 showed a less cohesive and more disorganized structure, which may be correlated with the lower shear force (1.06 kgf), and consequently the greater softness of the product. However, this lack of organization and compression resulted in high cooking loss (11.80%).

Comparing the observations found through microscopy with the results of shear force and cooking losses, it was concluded that there was a relationship between some properties and their respective microscopic structures. The best results were found for the treatment F2, indicating that the Fiber 600/30 (large particles) allowed the maintenance of a texture close to the control F1 (without fiber addition), and lower cooking loss when compared to the product containing fiber of smaller particle size (Fiber 600/30). Although the treatments F4 and F5 (containing fiber mixture) had lower cooking losses, there was an increase in hardness, which could harm the quality of the product.

4 Conclusion

Significant differences were observed ($p < 0.05$) for the physical properties of the developed burgers due to both the variations in the particle size and the different concentrations of wheat fiber. The best results were found for the F2 treatment, indicating that Fiber 600/30 (large particles) allowed the maintenance of a texture close to the standard F1 (without fiber addition) and lower cooking loss when compared to the product containing fiber of smaller particle size (Fiber 600/30). Comparing the observations obtained by microscopy with the results of shear force and cooking losses, it was concluded that there is a relationship between some properties and their respective microscopic structures.

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