International Food Research Journal 21(4): 1523-1528 (2014)

Journal homepage: *http://www.ifrj.upm.edu.my*

Technological quality of bread from rice flour with *Spirulina*

*Selmo, M. S. and Salas-Mellado, M. M.

Laboratory of Food Technology, School of Chemistry and Food, Federal University of Rio Grande, Alfredo Huch, 475, Rio Grande do Sul, Brazil

Article history

Received: 15 December 2013
Received in revised form: 11 February 2014
Accepted: 12 February 2014

Keywords

Bread
Rice
Spirulina,
Transglutaminase
Methylcellulose

Abstract

Aiming to improve the technological characteristics and test the preference of gluten free bread, destined principally for celiac population, an experimental design was carried out to define the levels of methylcellulose, transglutaminase and *Spirulina* in the formulation of rice flour bread. The experiment was conducted according to a 2^3 full factorial CCRD, using variables methylcellulose, transglutaminase and *Spirulina*. From the responses of technological quality of the brightness of the crumbs, specific volume and crumb firmness of bread three formulations of rice flour that were defined were evaluated technologically, chemically and sensorially by a preference test. Regarding crumb brightness, bread with higher content of *Spirulina* presented decreased brightness. The specific volume of the bread showed values in the range of 2.93 $mL.g^{-1}$ and 3.10 $mL.g^{-1}$ and crumb firmness between 316.25 g and 436.39 g. With an increasing concentration of *Spirulina* from 1% to 4%, there was a 20% increase in the protein content of rice flour bread. The sensory evaluation showed no difference in consumer preference between the loaves with 1% or 4% of *Spirulina*, from rice flour basis.

© All Rights Reserved

Introduction

Celiac disease (CD) is defined as a permanent intolerance to gluten, a protein found in cereals such as wheat, oats, rye and barley. CD affects genetically susceptible individuals, causing an inflammatory process that alters the lining of the small intestine, causing villous atrophy and malabsorption of nutrients (Nimmo, 2005). The gluten of wheat flour imparts unique viscoelastic properties to baked products. During the fermentation process, an elastic and resilient dough is required to withstand the pressure of the gases that form within the dough, thus providing the increased volume of the loaves (Goesaert *et al*., 2005).

Studies have been conducted aiming to replace the wheat flour by other ingredients capable of forming a protein network to retain the CO_2 produced during bread fermentation (Gallagher *et al.*, 2004; Gujral and Rosell, 2004; Sivaramakrishnan *et al.*, 2004; Clerici and El-Dash, 2006; Moore *et al.*, 2006; Marco and Rosell, 2008; Figueira *et al.*, 2011; Blanco *et al*., 2011). Rice is an option for the production of gluten-free products, since besides being a protein food free of enzyme inhibitors that impair the absorption of nutrients, it has a mild flavor, white color, hypoallergenic properties (Neumann and Bruemmer, 1997) and a high proportion of easily digestible carbohydrates. However, the dough made from rice flour has limited capacity to retain the gases formed during bread processing because it has no important viscoelastic properties, producing bread of low specific volume (Rosell and Marco, 2008). Accordingly, various substances may be used singly or in combination to assist in the formation of the network and thus improve the technological characteristics of the rice flour bread.

The enzyme transglutaminase has been studied due to its ability to form a protein network, through the promotion of cross-links between glutamine and lysine residues, converting soluble proteins into insoluble polymers (Larré *et al*., 2000; Moore *et al*., 2006; Stefano *et al.*, 2008). This formed network can make the dough have more elasticity, improving the ability to retain gas during fermentation. However, combined use with gums or hydrocolloids would still be necessary to improve the loaf volume and compensate for the absence of the gluten network (Clerici and El- Dash, 2006; Stefano *et al*., 2008).

The hydrocolloids, also called gums, are long chain polymers of high molecular weight soluble in water, capable of increasing the viscosity of the system, they help in the structure and in some cases form gels (Gharibzahedi *et al*., 2012). Gums or hydrocolloids are widely used in food technology and are aimed at improving the texture, the retardation of starch retrogradation and increasing moisture retention. Among the functional effects of hydrocolloids in bakery products there is the possibility of acting in modification of the rheology of the dough, increasing the quality of the finished products. They are often used as substitutes for gluten

*Corresponding author.
Email: *mselmo@ig.com.br*

in gluten-free bread (Roberts *et al*., 2012). The reason for which gel formation can be important in gluten free products is that gelation involves the formation of a three-dimensional network. Hydrocolloid gels are viscoelastic, just as the gluten in the dough made from wheat flour (Bemiller, 2008).

Spirulina is a microalgae that is uses in human food for having high protein, vitamin and mineral contents (Ambrosi *et al*., 2008). According to Cauvain and Young (2009), the addition of a protein source is useful in adjusting the dough when one prepares gluten free bread, preventing the collapse of the product. Thus, in the preparation of gluten free bread using rice flour, *Spirulina* may be indicated to assist in the forming of a network improving the volume of the bread. The objective of this work was to improve the technological characteristics of rice flour bread by adding methylcellulose, transglutaminase and *Spirulina* and to test consumer preference among the best bread produced.

Material and Methods

Raw materials and ingredients

The rice flour used was provided by the Cerealle Company (moisture 6.8%, 8.0% protein, 0.5% lipid, 0.7% ash and 84% carbohydrate), Ascorbic Acid P.A. from Synth, Transglutaminase Activa WM (81-135 U/g) Enzyme given by Ajinomoto Co., Hydrocolloid Methylcellulose Methocel A4M®, and *Spirulina* microalgae strain LEB -18 (protein 53.1%, ash 12.2%, 10.2% lipids , 24.5% carbohydrates), dried and milled to a particle size of 80 mesh obtained in the laboratory of Biochemical Engineering, Federal University of Rio Grande, RS, Brazil. The other ingredients used in the preparation of bread were purchased in the local market of Rio Grande.

Bread preparation

The amounts of ingredients used for the dough of the bread, on the basis of 100% rice flour were: 120% water, 2% salt, 5% refined sugar, 1.5% dry yeast, 6% oil soybean, 90 ppm of ascorbic acid and methylcellulose, transglutaminase and *Spirulina* according to experimental design levels. For the processing of bread, the following procedures were standardized: weighing the ingredients, mixture of the dry ingredients, admixing of the liquid ingredients for 10 minutes on low speed using a planetary mixer (KitchenAid) ingredients; fermentation in an oven (Quimis) at 30°C for 60 minutes (first fermentation), dividing the dough into pieces of 175 g in metal tins of 13.3 x 5.5 cm smaller base, 15.5 x 7.4 cm upper larger base with a height of 4.5 cm; fermentation in an oven at 30°C for 55 minutes (second fermentation), cooking in an electric oven (model Diplomat, Fischer) at 200°C for 20 minutes, removal of the bread from the tins and cooling.

To study the effect of addition of methylcellulose, transglutaminase and *Spirulina* in the characteristics of bread, 2^3 full factorial CCRD experimental design (Box and Wilson, 1951) was applied with three central points, totaling 17 trials. The independent variables and levels used were: methylcellulose (0.8% to 2.2%), transglutaminase (0.2% to 1.0 %) and *Spirulina* (1.0% to 4.0%).

Technological analysis of the experimental design

The response variables evaluated were: brightness (L^*) of the crumbs and specific volume ($mL.g^{-1}$) and crumb firmness (g). The brightness (L^*) of the bread crumb was determined using a colorimeter Minolta®, model CR400. The bread were weighed on an analytical balance and the apparent volume of bread determined by the AACC method 10-05 (2000). The calculation of specific volume was conducted by the ratio between the apparent volume and mass of the baked bread ($mL.g^{-1}$). The evaluation of crumb firmness of rice flour bread was performed according to the method of AACC (2000) (74-09) using the Texture Analyzer TA.XT plus using the Exponent software. The effects of the independent variables on the responses, brightness of the crumb, specific volume and crumb firmness of bread, were analyzed using Response Surface Methodology. Using the STATISTICA 7.0 (Statsoft, USA), the non-significant terms ($p \geq 0.10$) were discarded, obtaining a more appropriate adjusted model to describe the effects of the independent variables on the responses analyzed.

Evaluation of rice flour bread A, B and C

Based on the responses of the experimental design three formulations of rice flour bread with better technological characteristics, denominated Bread A, Bread B and Bread C were chosen, they were evaluated according to their technological characteristics of brightness of the crumb, specific volume and crumb firmness using the same methodology used in the experimental design. The following proximal composition analyses of the bread were made: moisture, determined at 105°C at constant weight (AOAC, 2000); ash determined in oven at 550°C (AOAC, 2000); protein performed by micro-Kjeldahl method (N x 5.95) (AOAC, 2000) and lipids by Soxhlet method (AOAC, 2000) and the estimated total carbohydrates by difference.

Breads were also evaluated by a ranking by preference sensory test, according to the Adolfo Lutz Institute (2008). The sensory evaluation was approved by the Health Research Ethics Committee (CEPAS) of the Federal University of Rio Grande, RS, Brazil, through opinion report n° 130/2012. To evaluate breads, fifty consumers were invited to participate in the event. Randomly coded bread samples were presented to the judges so that they could rank in decreasing order of preference, ie, the most preferred (1) to least preferred (3).

Statistical analysis

The differences between the values of technological characteristics and proximate composition of bread A, B and C were evaluated by analysis of variance (ANOVA) and means were compared by Tukey test at 5% significance. The result of sensory evaluation was calculated as the sum of the rankings obtained from the judges for each sample using the Friedman test at 5% significance and compared with values of Newell and MacFarlane table (Adolfo Lutz Institute, 2008).

Results and Discussion

Effect of the addition of methylcellulose, transglutaminase and Spirulina on the technological characteristics of bread

All independent variables presented a significant effect ($p \leq 0.10$) on the specific volume of bread, allowing to establish a mathematical model, under the conditions studied. The results represented by the contour curves of Figure 1 show that increasing the content of methylcellulose and Spirulina results in bread with increased specific volume, when the level of transglutaminase is low. The specific volume is the most important measure for the ability of flour to expand and retain the gas within the bread dough during baking, thus methylcellulose levels greater than 1.5%, *Spirulina* greater than 3.4% and transglutaminase less than 0.36% positively affect the volume of the bread.

Figueira *et al.* (2011) observed that the addition up to 4% of *Spirulina* on rice based flour caused no significant difference in the volume of rice flour bread, when the concentrations of hydrocolloid and transglutaminase were 2.0% and 0.5%, respectively. In contrast, in this study, the concentration of *Spirulina* increased specific volume of the bread. Studying the effect of transglutaminase and protein isolates in gluten free bread, Shin *et al.* (2010), found a specific bread volume of 1.76 $mL.g^{-1}$ for rice flour when transglutaminase was added and the specific volume increased to 1.93 $mL.g^{-1}$ when soy protein isolate was added.

In crumb firmness only the quadratic term of the methylcellulose concentration variable and interaction of *Spirulina* concentration with the concentration of transglutaminase were statistically significant ($p < 0.10$). As shown by the contour curves of Figure 2, the greatest softness of the bread was obtained from rice flour at the methylcellulose midpoint level (1.5%), while the concentrations of transglutaminase and *Spirulina* had an antagonistic effect. When the concentration of transglutaminase was increased and *Spirulina* was reduced, there was improvement in the softness of the bread in the same way that when an increase in *Spirulina* concentration and transglutaminase decrease.

In their study, Figueira *et al.* (2011) showed that the level of 2.0% HPMC and 0.5% transglutaminase, the addition of *Spirulina* contents of up to 4% increased the bread softness, whereas with the addition of 5% *Spirulina* decreased the softness of the bread. In this study, something similar occurred, because when the methylcellulose content was kept at 1.5 % and the *Spirulina* concentration increased an increase in softness of bread was also seen, provided that the transglutaminase level was reduced.

For the variable crumb brightness, methylcellulose and *Spirulina* concentration variables were statistically significant ($p \leq 0.10$), while the addition of transglutaminase was not significant. According to the results shown in the contour curve of Figure 3, the increased crumb brightness was due to the increase in content of methylcellulose and decreased content of *Spirulina.* Thus, larger amounts of methylcellulose and smaller amounts of *Spirulina* make the rice flour bread whiter. The progressive addition of *Spirulina* causes a decrease in brightness, that is, it makes the bread crumb darker due to dark green microalgae. To obtain an estimate of darkening, the L^* (brightness) coordinate has often been used, thus the higher its value, the lighter the sample and therefore less browning. According to Jackix and Cohen (2005), the L^* values vary from 0 (black) to 100 (white) and usually below values 50 ($L^*<50$) are considered to be dark colors.

Figueira *et al.* (2011) also achieved a reduction of the brightness of the crumbs with an increase in the concentration of added *Spirulina.* Increasing the concentration of methylcellulose affects the brightness of the bread making them lighter probably due to better retention of oxygen inside the dough during kneading, which causes greater uniformity and fineness of cells in the crumb.

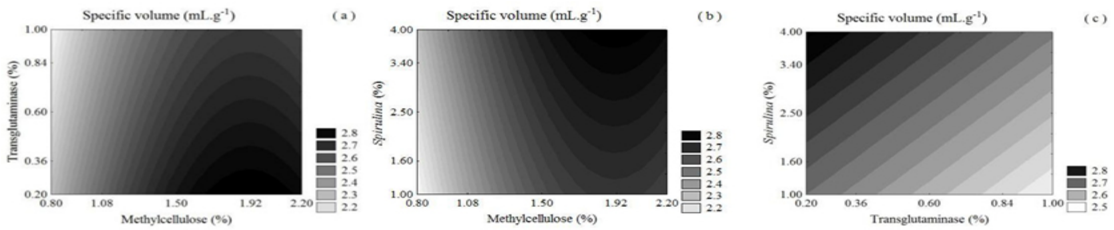

Figure 1. Contour curves for the specific volume (mL.g^{-1}) of bread due to the concentration of transglutaminase and methylcellulose (a), concentration of *Spirulina* and methylcellulose (b) and concentration of *Spirulina* and transglutaminase (c).

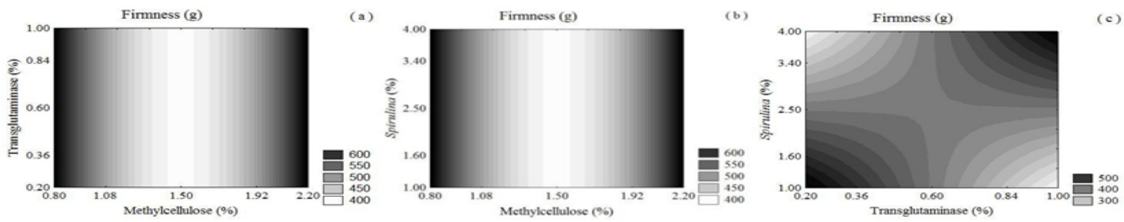

Figure 2. Contour curves for the firmness (g) of bread crumb due to the concentration of transglutaminase and methylcellulose (a), concentration of *Spirulina* and methylcellulose (b) and concentration of *Spirulina* and transglutaminase. (c).

Levels of independent variables for Bread A, B and C

The levels of the variables methylcellulose, transglutaminase and *Spirulina* defined by the experimental design responses and chosen for the preparation of bread with better technological characteristics were:

Bread A - 2.2% methylcellulose; transglutaminase 0.2% and 4.0% *Spirulina*

Bread B - 1.5% methylcellulose; transglutaminase 0.2% and 4.0% *Spirulina*

Bread C - 2.2% methylcellulose; transglutaminase 0.6% and 1.0% *Spirulina*

Technology evaluation and proximal composition of bread A, B and C

Table 1 shows the means and standard deviations of the characteristics of crumb color, specific volume, crumb firmness and proximate composition of bread A, B and C. The hydrocolloids are important in a gluten-free mass, since they retain the gases produced during fermentation improving the specific volume of the bread. Bread C, prepared with the lowest concentration of *Spirulina* and 0.6% transglutaminase did not differ significantly ($p \geq 0.05$), from the other breads. This shows that when the concentration of *Spirulina* is smaller, the transglutaminase should be higher in order to maintain the specific volume of the bread. A similar result was obtained by Figueira *et al.* (2011) who obtained specific volume of 3.03 mL.g^{-1} when they used 2% *Spirulina*, 2% HPMC and 0.5% of the transglutaminase in rice flour bread. Studying the effects of protein and transglutaminase in the preparation of gluten-free rice bread, Shin *et al.* (2010), found a specific bread volume of 1.76 mL.g^{-1} of rice flour with transglutaminase and when soy protein isolate was added the volume increased to 1.93 mL.g^{-1}. Moore *et al.* (2006) concluded in their work on the use of transglutaminase in gluten-free bread containing added protein sources, that it is possible to form protein network of gluten-free bread with addition of transglutaminase, but the efficiency depends on the structure of the enzyme protein source used and the level of enzyme used. In this study, concentrations of 0.2% to 0.6% of enzyme transglutaminase were effective to improve the specific volume of rice flour bread at *Spirulina* concentrations of 1% to 4%.

Bread A presented the highest crumb firmness, differing significantly ($p \leq 0.05$) from the others, so bread B and C were softer, besides presenting higher specific volume. There is a relationship between the specific volume and firmness of bread, which occurs due to greater compression of the crumbs when the specific volume is smaller, causing increased resistance to deformation and consequent decrease in softness of the bread. Figueira *et al.* (2011) obtained rice flour bread with 4% *Spirulina* much softer than that in this study, obtaining firmness value of 277.39 g. According to Quaglia (1991), the hydrocolloid methylcellulose has the function of increasing the water absorption and improving the consistency of bread crumb of low gluten content.

The values of brightness (L*) of the bread crumbs of A and B are very similar to the value of 33.57 which was found by Figueira *et al.* (2011) for

Table 1. Technological characteristics and proximate composition of breads A, B and C

Characteristics and composition	Bread A	Bread B	Bread C
Specific volume (mL.g^{-1})	2.93 ±0.07^{b}	3.10 ±0.08^{a}	3.03 ±0.02^{ab}
Firmness (g)	436.39 ±31.83^{a}	326.55 ±39.80^{b}	316.25 ±42.05^{b}
L * crumb	34.59 ±2.53^{b}	33.89 ±1.03^{b}	53.21 ±0.83^{a}
Moisture (%)	45.67 ±0.59^{a}	45.02 ±0.36^{a}	45.41 ±0.53^{a}
Ash (%) *	2.57 ±0.05^{a}	2.52 ±0.02^{a}	2.30 ±0.07^{b}
Protein (%) *	8.62 ±0.35^{a}	8.69 ±0.29^{a}	7.19 ±0.11^{b}
Lipids (%) *	4.69 ±0.50^{a}	4.17 ±0.13^{a}	4.43 ±0.23^{a}
Carbohydrates (%) *	84.12 ±0.89^{a}	84.62 ±0.43^{a}	85.98 ±0.36^{a}

Different superscript letters in a row are significantly different by Tukey test ($p < 0.05$).*Dry basis.

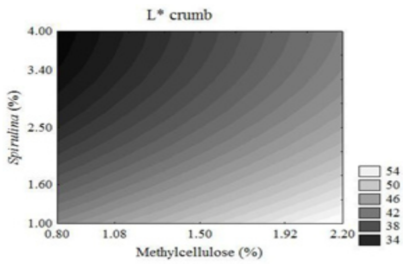

Figure 3. Contour curve for brightness (L*) of bread crumb due to the concentration of methylcellulose and *Spirulina*

the bread with 4% *Spirulina*, while bread C showed a higher value indicating that the reduction of the concentration of *Spirulina* brightens the bread. The protein content of the Bread A and B was significantly higher than that of the bread C due to the increased content of *Spirulina* present in bread. The increase in the amount of 1% *Spirulina* (bread C) to 4% (bread A and B) increased by 20% the protein content of the bread. Figueira *et al.* (2011) found similar results in protein content: 9.33% in bread with 4% *Spirulina* and 7.83% in bread with 2% *Spirulina.* The ash content was also significantly increased due to a high mineral content of *Spirulina.*

Sensory evaluation

The results of the rice flour bread ranking - preference test (Table 2) show that the preference of the judges was the bread with lower content of *Spirulina*, which gives a lighter crumb color, though it did not differ statistically from bread A, which has a higher content of *Spirulina*. There was a statistically significant difference only between bread B and C. Of the 50 judges, 40% preferred bread C, 38% bread A and only 22% preferred bread B.

The judges, in their comments, made reference to the similar flavor of the bread, the differentiated bread texture of B and different color of bread C compared with others. According to Bobbio and Bobbio (1992), the consumption of a food, known or not by the consumer, depends in the first instance, on its color and its appearance, since when a consumer comes in contact with food, the color and appearance are the first two sensations that reach him, and is what will lead to the acceptance, rejection or indifference.

Table 2. Ranking preference test result of rice flour bread

Bread	Punctuation*
A	94 ^{ab}
B	115 ^{a}
C	91 ^{b}

*Different superscript letters in a column are significantly different by Friedman test ($p < 0.05$).

Conclusion

There was improvement in the technological characteristics of rice flour bread, using methylcellulose, transglutaminase and *Spirulina*, allowing one to define formulations with good technological, chemical and sensory characteristics. The hydrocolloid methylcellulose was important to improve the characteristics of the specific volume and crumb firmness at levels above 1.5%. The enzyme transglutaminase was effective in promoting the formation of protein network in concentrations from 0.2 % to 0.6 % depending on the amount of *Spirulina* added. The increase in concentration from 1% to 4% of *Spirulina* in the formulations of rice flour bread did not affect the preference of the judges and increased protein content by 20%, and improving the specific volume and crumb firmness.

References

Ambrosi, M.A., Reinehr, C.O., Bertolin, T. E., Costa, J.A.V. and Colla, L.M. 2008. Propriedades de saúde de *Spirulina* spp. Revista de Ciência Básica Aplicada 29 (2): 109-117.

American Association of Cereal Chemists (AACC). 2000. Approved Methods of the AACC. 10th edition. American Association of Cereal Chemists, St. Paul, MN.

Association of Official Analytical Chemists Official Methods of Analysis (AOAC). 2000. Official Methods of Analysis, 17ª Edição, Washington, D. C., CD-ROM.

Bemiller, J.N. 2008. Hydrocolloisds. In: EA Arendt and F Dal Bello (eds), Gluten-free cereal products and beverages, p.203-215. Elsevier, Oxford, UK.

Blanco, C.A., Ronda, F., Pérez, B. and Pando, V. 2011. Improving gluten-free bread quality by enrichment with acidic food additives. Food Chemistry 127: 1204–1209.

Bobbio, F.O. and Bobbio, P.A. 1992. Química do Processamento de Alimentos. São Paulo: Livraria Varela, 222p.

Box, G.E.P. and Wilson, K.B. 1951. On the experimental attainment of optimum condition. Journal of Royal Statistical Society. Series B 13: 1-9.

Cauvain, S.P. and Young, L.S. 2009. Tecnologia da panificação. Barueri, SP: Manole.

Clerici, M.T.P. S. and El-Dash, A.A. 2006. Farinha extrusada de arroz como substituto de glúten na produção de pão de arroz, Archivos Latinoamericanos de Nutrición 56 (3): 288-298.

Cohen, K.O. and Jackix, M.N.H. 2005. Estudo do liquor de cupuaçu. Revista Ciência e Tecnologia de Alimentos Campinas 25 (1): 182-90.

Figueira, F., Crizel, T.M., Silva, C.R. and Salas-Mellado, M.M. 2011. Pão sem glúten enriquecido com a microalga Spirulina platensis, Brazilian Journal of Food Technology, Campinas 14 (4): 308-316.

Gallagher, E., Gormley, T.R. and Arendt, E.K. 2004. Recent advances in the formulation of gluten-free cereal-based products. Food Science and Technology 15: 143-152.

Gharibzahedi, S.M.T., Mousav, I.S.M., Hamedi, M., Khodaiyan, F. and Razavi, S.H. 2012. Development of an optimal formulation for oxidative stability of walnut-beverage emulsions based on gum arabic and xanthan gum using response surface methodology, Carbohydrate Polymers 87: 1611– 1619.

Goesaert, H., Brijs, K., Veraverbeke, W.S., Courtin, C.M., Gebruers, K. and Delcour, J.A. 2005. Wheat flour constituents: how they impact bread quality, and how to impact their functionality. Trends in Food Science and Technology 16 (1): 12-30.

Gujral, H.S. and Rosell, C.M. 2004. Improvement of the breadmaking quality of rice flour by glucose oxidase. Food Research International 37: 75-8-1.

Instituto Adolfo Lutz . 2008. Métodos físico-químicos para análise de alimentos, São Paulo: Instituto Adolfo Lutz, 1ª edição digital, p.1020. Available from *http://www.crq4.org.br/sms/files/file/analisedealimentosial_2008.pdf* in 30/10/2013.

Larré, C., Denery-Papini, Y., Popineau, G., Deshayes, C., Desserme, C. and Lefebvre, J. 2000. Biochemical Analysis and Rheological properties of Gluten Modified by Transglutaminase. Cereal Chemistry 77 (2): 121-127.

Marco, C. and Rosell, C.M. 2008. Effect of different protein isolates and transglutaminase on rice flour properties. Journal of Food Engineering, Meppel 84 (1): 132-139.

Moore, M.M., Heinbockel, M., Ulmer, H.M. and Arent, E.K. 2006. Network formation in glutenfree bread with application of transglutaminase. Cereal Chemistry 83 (1): 28-36.

Neumann, H. and Bruemmer, J.M. 1997. Investigations with the production of gluten-free bread and roll specialities. Getreide Mehl und Brot 51: 50-55.

Nimmo, M. 2005. Celiac disease: an update with emphasis on diagnostic considerations. Laboratory Medicine 36(6): 366–369.

Quaglia, G. 1991. Ciência y tecnologia de la panificacion Zaragoza, Espanha : Acribia.

Roberts, K.T., Cui, S.W., Chang, Y.H. and Graham, T. 2012. The influence of fenugreek gum and extrusion modified fenugreek gum on bread, Food Hydrocolloids 26: 350-358.

Rosell, C.M. and Marco, C. 2008. Rice. In: E.A. Arendt and F. Dal Bello (eds), Gluten-free cereal products and beverages, p.81-100, Elsevier, Oxford, UK.

Shin, M., Gang, D.O. and Song, J.Y. 2010. Effects of protein and transglutaminase on the preparation of gluten-free rice bread. Food Science and Biotechnology 4 (19): 951-956.

Sivaramakrishnan, H.P., Senge, B. and Chattopadhyay, P.K. 2004. Rheological properties of rice dough for making rice bread. Journal of Food Engineering 62: 37-45.

Stefano, R., Dal Bello, F. and Arendt, E.K., 2008. Microstructure, fundamental rheology and baking characteristics of batters and breads from different gluten-free flours treated with a microbial transglutaminase. Journal of Cereal Science 48 (1): 33-45.