International Food Research Journal 21(4): 1597-1602 (2014)

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

Use of transglutaminase, soybean waste and salt replacement in the elaboration of trout (*Oncorhynchus mykiss*) meatball

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Article history

Received: 7 October 2013
Received in revised form: 22 January 2014
Accepted: 23 January 2014

Keywords

Rainbow trout
Centesimal composition
Sensory analysis
Penalty analysis

Abstract

The development of new products based on fish with non-commercial size is an alternative to add value, conquer new consumer markets and consequently, increase this matrix consumption. The objective of this study was to assess the physicochemical and sensory parameters of meatballs prepared with non-commercial size Rainbow Trout fillets, waste added of transglutaminase, soy protein and 25% replacing salt. The transglutaminase can be used to modify the functional properties of food protein, in addition to gelation capability, thermal stability and water-holding capacity and soy protein presents several technological properties that are very importante in characterization in the production of texturized food. The salt replacement has been studied because of consumers search for healthy products and an alternative strategy, such as sodium chloride replacement by potassium chloride have been implemented because the potassium chloride has similar properties to sodium chloride and can be used as a substitute without losing functionality. The transglutaminase and soy protein associated to transglutaminase improved the meatball texture; however the soy protein provided bitter taste and less juiciness. A reduction in sodium content can be achieved without impairing the product physicochemical and sensorial quality replacing sodium chloride by potassium chloride.

Introduction

Fish is the food matrix of animal origin with the highest production and industrialization worldwide. However in Brazil, due to cultural aspects and lack of government incentives to boost seafood industries and consumption, percapita consumption is low (9 kg/per capita/year), thus justifying the constant search for new products aimed at diversification and the conquest of new markets to increase this matrix consumption in the country (Bery *et al*., 2012; MPA, 2012; FAO, 2012).

To obtain diversified, healthy, inexpensive and sustainable seafood products, waste solids from fish industrialization must be exploited. These wastes have a high nutritional value, because they are rich in protein and omega-3 fatty acids, and can be used to prepare foodstuffs for human consumption (Feltes *et al*., 2010).

The preparation of restructured products is an alternative method to increase the profit of cuts with low commercial value (Castro-Briones *et al*., 2009). In the same way, some ingredients and additives such as binders (soy protein), enzymes and sodium chloride could be used in order to improve the technological and sensory properties (López-López *et al*., 2010).

Soy protein presents several technological properties such as emulsification, fat and water absorption and texture improvement, important characteristics in the production of texturized food (Wang *et al*., 2010; Guerrero and La Caba, 2010; Schmiele *et al*., 2013). In addition, soy protein is considered a functional food capable of acting as modulator of metabolic processes and in the prevention of the onset of degenerative diseases (Boye *et al*., 2010; Granato *et al*., 2010).

The transglutaminase enzyme (MTG) is frequently used in the food industry in the preparation of restructured products. This enzyme promotes intra and intermolecular bonds between proteins (Huang *et al*., 2010), improving product texture and taste, making them well accepted by the consumer and increasing its commercial value (Feltes *et al*., 2010; Ferreira *et al*., 2012). The ability of transglutaminase to modify the functional properties of protein foods has been one of the most innovative uses of enzymes in food technology from the past decade (Lorenzen, 2007).

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Another aspect that currently increases the marketing value of food products is the reduction of sodium chloride, widely used in meat products, because of several technological and sensorial properties important to industrialized products such as preservation action and increasing water retention capacity of proteins thus improving texture and consequently sensory attributes (Doyle and Glass, 2010; Taormina, 2010). Although this ingredient is directly related to high blood pressure, there is a strong resistance of the food industries to reduce sodium content in meat products, because this reduction causes sensory changes (Aliño *et al*., 2010; Guallar-Castillón *et al.*, 2013). Thus, alternative strategies, such as sodium chloride replacement by potassium chloride have been implemented. Potassium chloride has similar properties to sodium chloride and can be used as a substitute without losing functionality (Nascimento *et al.*, 2007), but this replacement must be carefully made because potassium chloride tastes bitter (Aliño *et al.*, 2010).

The development of a new product based on non-commercial size fish using transglutaminase as binder (Moreno *et al.*, 2009), adding soy protein due to its functional and technological effects (Boye *et al.*, 2010) and reducing sodium (Cardoso *et al.*, 2010) may be an alternative in the seafood industry. In this context, the objective of this work is to assess the influence of adding soy protein and transglutaminase and partially replacing sodium chloride by potassium chloride in the physicochemical and sensory characteristics of rainbow trout meatballs prepared from non-commercial size fillets

Materials and methods

Raw material

The trouts used did not reach an adequate commercial weight and thus they were treated as waste and used to prepare the restructured food. The weight of fillets acquired was approximately 80 grams each and the commercial weight of rainbow trout is 150 grams (Macedo-Viegas *et al.*, 2002), so these fillets that did not achieve ideal weight would be discardedThe rainbow trouts (*Oncorhynchus mykiss*) were captured in tanks, and after removing the head were eviscerated, manually sliced and frozen. Meat samples were ground in a meat grinder using a plate with 3 mm diameter holes.

Five different restructured trout formulations, with different levels of soy protein and MTG besides partial replacement of sodium chloride by potassium chloride, were prepared as a follow: T1 – starch addition (control); T2 – MTG addition (1%); T3 – soy protein addition (4%); T4 - soy protein addition (4%) and MTG addition (1%); T5 - soy protein addition (4%), MTG addition (1%) and partial replacement of salt (75% NaCl/ 25% KCl). Starch was used when soy protein and MTG were not used. Dehydrated seasonings were added to the raw material in technically important sequence and mixed to form a homogenous mass. The seasoning percent was the same for all the treatments and is shown in Table 1. The fish meatballs were manually shaped, weighing approximately 30 g each, packed in expanded polystyrene trays and stored approximately at -25°C.

Table 1. Base formulation of the meatballs trout

Ingredientes	T1	T2	T3	T4	T5
Pulp	91,5	94,5	91,5	90,5	90,5
Sugar	0,2	0,2	0,2	0,2	0,2
Ascorbic acid	0,2	0,2	0,2	0,2	0,2
Salt	1,5	1,5	1,5	1,5	1,125
Garlic	1,0	1,0	1,0	1,0	1,0
Soy Protein	---	---	4,0	4,0	4,0
Onio	1,0	1,0	1,0	1,0	1,0
Transglutaminase	---	1,0	---	1,0	1,0
Chive	0,6	0,6	0,6	0,6	0,6
Starch	4,0	---	---	---	---
Potassium chloride	---	---	---	---	0,375

*Refers to the ingredients in 100 g of the final product
**T1 starch addition, T2 MTG addition, T3 soy protein addition, T4 soy protein and MTG addition and T5 soy protein addition, MTG addition and partial replacement of salt.

Centesimal composition

The centesimal composition was determined according to the analytical norms of the Association of Official Analytical Chemists (AOAC, 2005). Aliquots were removed to determine the following parameters: moisture, calculated by the water loss when the sample is dried to constant weight (oven at 105ºC); ether extract, using a sohxlet extractor and petroleum ether as solvent; crude protein, determining total nitrogen by Kjeldahl digestion process, and fixed mineral residue by complete incineration of organic compounds (muffle furnace at 550ºC).Carbohydrates were quantified by the Dinitrosalicylic Acid Method (DNS) described by Miller (1959).

Sensory evaluation

Acceptance test and scale of ideal

The sensory evaluation comprised an acceptance test using the hedonic scale structured in 9 points having as end-anchors "1 – dislike extremely and 9 = like extremely" (taste, smell, texture, bitter, overall impression) as described by Lee (2011). The test was performed with 100 adult non-trained panelists, aged from 20 to 66 years (being 57 female and 43 male). Each panelist received, at the beginning of the test, an evaluation sheet containing a 9 point hedonic scale (9 = like extremely; 5 = neither like nor dislike; 1 = dislike extremely). In addition to the acceptance test, attributes such as juiciness, firmness, salty taste, seasoning intensity and bitter taste intensity were measured using an hedonic scale where 1 = extremely less juicy, firm, salty, seasoned or bitter; 5 – ideal; 9 = extremely more juicy, firm, salty, seasoned or bitter, according to Cadot *et al*. (2010).

Just about right

Using the results of the hedonic scale of ideal, the Just- About-Right (JAR) was performed. This methodology directly approaches the measure of the deviation from ideal levels of a specific attribute, using scales where the end anchors are “extremely weak” and “extremely strong” and a center point which is the ideal (Chambers *et al.*, 1996). The JAR is usually expressed through the percent of panelists that consider the level of the attribute as too much, too little or just-about-right (JAR). Together with JAR a penalty analysis was conducted through the deviations from ideal (Cavitt *et al*., 2005).

The tests were performed in closed cabin and white lighting, with the samples placed under the counter and labeled with three random numbers each. A Variance Analysis and Tukey’s multiple comparison test ($p < 0.05$) were applied to the results using XLSTAT 2012.6.08 version software (Addinsoft, Paris, França).

Results and Discussion

Centesimal composition

Moisture values varied from 69.24 to 73.60%, protein from 18.6 to 20.8%, lipids from 2.45 to 3.05%, carbohydrates from 1.25 to 4.92 and ashes from 2.1to 3.1%. These results are shown in Table 2. The highest moisture content was observed in the sample with addition of MTG. This result may be related to the transglutaminase ability in promoting protein cross-linking conferring properties such as thermal stability and water retention (Huang *et al*., 2010).

The results showed that T1 (starch addition) presented the smallest amount of protein among all the treatments, followed by T2 (MTG addition). This is justified by the absence of texturized soy protein in those formulations. Treatments T3, T4 and T5, with soy protein addition, presented a significant increase of protein values when compared with the control (T1) that had starch added. However, moisture increased only in T2 (MTG addition) and T4 (MTG and soy protein addition) when compared to the control. Although soy protein and transglutaminase were added to T5, it did not present significant moisture increase, probably because of partial replacement of sodium chloride, thus reducing the water retention capacity. There was no significant difference ($p > 0.05$) in relation to ash, lipids and carbohydrates among treatments

Table 2. Results of physicochemical analysis of meatballs trout

Treatments/ Analyzes	T1	T2	T3	T4	T5
Moisture	71,23^{a}	73,60^{b}	70,22^{a}	69,24^{c}	71,53^{a}
Protein	18,60^{a}	19,50^{a}	20,60^{b}	20,80^{b}	20,60^{b}
Lipid	2,45^{a}	2,65^{a}	2,50^{a}	3,05^{a}	2,45^{a}
Carboydrate	4,92^{a}	1,25^{a}	3,78^{a}	3.81^{a}	3.32^{a}
Ashes	2,80^{a}	3,00^{a}	2,90^{a}	3,10^{a}	2,10^{a}

[*]T1 starch addition, T2 MTG addition, T3 soy protein addition, T4 soy protein and MTG addition and T5 soy protein addition, MTG addition and partial replacement of salt.

Table 3. Sensory attributes and meatballs acceptance

Treatments/ Analyzes	T1	T2	T3	T4	T5
Appearance	7,24^{a}	7,22^{a}	7,12^{a}	7,07^{a}	7,04^{a}
Aroma	7,13^{a}	7,04^{a}	6,99^{a}	6,91^{a}	6,89^{a}
Flavor	6,86^{a}	6,58^{a}	6,55^{a}	6,46^{a}	6,35^{a}
Texture	6,81^{a}	6,58^{ab}	6,43^{ab}	6,35^{ab}	6,14^{b}
Global Impression	6,97^{a}	6,80^{a}	6,70^{a}	6,65^{a}	6,59^{a}

[*]T1 starch addition, T2 MTG addition, T3 soy protein addition, T4 soy protein and MTG addition and T5 soy protein addition, MTG addition and partial replacement of salt.

Acceptance test

The results of the sensory attributes and the acceptance of the 5 treatments of Rainbow Trout meatballs are shown in Table 3. Treatment T1 presented the best acceptance in relation to all the attributes. This result can be explained because starch has texturing, thickening, stabilizing, and water and fat linking characteristics that are well accepted by the consumers (Pedroso and Demiate, 2008).

There was no significant difference of the attributes appearance, smell, taste and overall impression. Regarding the texture attribute, T1 presented a significant difference ($p < 0.05$) from T5, showing that texture improvement is more efficient with starch than with soy protein and transglutaminase. There was no significant difference between treatments T2, T3 and T4 when compared to T5, thus proving that partial replacement of sodium chloride by potassium chloride did not alter the texture in those treatments. Different results have been reported on the effects of sodium chloride on the texture of meat products. Gou *et al.* (1996), found no significant difference in the attribute texture of fermented sausages with partial replacement of sodium chloride by potassium chloride, Armenteros *et al.* (2012) also found no significant difference when 50% sodium chloride was replaced by potassium chloride in cured and salted hams, similar results to those obtained in the present study. On the other hand, Matulis *et al.* (1995) observed in that same product a softer texture when sodium chloride was reduced from 2.5% to 1.5%, as well as Costa-Corredor *et al.* (2009), who verified that cured hams prepared with NaCl reduction were more tender. According to Matulis *et al.* (1995) the differences reported in literature may be caused by pH, animal species or heat treatment, factors that influence the properties of meat products.

In general, all treatments presented good acceptance of the assessed attributes, with appearance obtaining the highest values and texture the lowest.

Just- about- right

In the Just-About-Right (JAR) test, all the treatments were assessed as less juicy than ideal, but only T3 presented a significant difference in relation to JAR. Regarding firmness only T4 was assessed as significantly firmer ($p < 0.05$) than ideal. Saltiness was higher in T2 and T4 with T2 significantly ($p < 0.05$) higher than JAR. The attributes seasoning and bitter taste although with statistically different values in T2, T3 and T5, were JAR when compared to the ideal.

Statistical analysis

In JAR methodology, overall impression is considered to allow relating the deviations from ideal through penalty analysis (Xiong and Meullenet, 2006; Ares *et al*., 2009; Plaehn, 2013).

Penalty test

Penalty analysis was conducted comparing the general rankings of the attributes given by the tasters that assessed the product and characterized how much the product acceptance was influenced by a particular attribute being different from ideal (Plaehn, 2013). By analyzing the deviations from ideal JAR of each attribute can be related through penalty test (Cavitt *et al*., 2005). This is accomplished by analyzing sensor data to identify attributes that are not ideal and influence on product quality (Caddot *et al.*, 2010).

The attribute juiciness was penalized in T3, which received the smallest score as a function of soy protein addition. The result obtained in the present study is in agreement with the result found by Hautrive et al. (2008), who reported that ostrich hamburger prepared with soy protein can have different sensory quality because water loss during cooking can impair the tenderness and juiciness of meat.

The attribute firmness was penalized in treatments T2 and T4, by 42% and 51% consumers respectively. The consumers observed greater firmness than JAR when transglutaminase and transglutaminase associated to soy protein were added. Similar results were described by Nonaka *et al*. (1996), who observed that the addition of transglutaminase and soy protein, besides maintaining soft texture, increased the gel strength in restructured meat products, seafood and fish balls as described by Soeda (2003) who concluded that the gel strength of soy protein was higher when MTG was added. The firmness attribute was not penalized in T5, where there was salt partial replacement. A similar result was observed by Choi *et al.* (1987), who concluded that there was no statistical difference in compression test when comparing Frankfurt type sausages replacing 1.5 and 3.0% NaCl, and by Armenteros *et al.* (2009), who studied salted smoked loins with 50% replacement of NaCl by KCl, and did not observe a significant difference in any attribute of sensory analysis.

Regarding the bitter taste attribute, T3, T4 and T5 were penalized. These treatments had soy protein added, and the perception of bitter taste is more accentuated. The same was described by Piazzon-Gomes *et al*. (2010) when they compared Minas frescal cheese with Minas frescal cheese added of water soluble soybean extract powder (PS-60). These authors observed that the bitter taste was more intense in the Minas frescal cheese with PS-60 than in the traditional Minas frescal cheese. The soybean bitter taste is attributed to saponins and isoflavons (LIU, 1997). This penalty may have influenced the perception of seasoning by the panelists, because this attribute was also penalized in the above mentioned treatments.

The salty attribute was only penalized in T5, where there was partial replacement of sodium chloride. A similar result was described by Nascimento *et al*. (2007) where the replacement of NaCl by KCl (75/25%), although near the ideal, proved to impact the perception of salty taste. On the other hand, different results were described by Durack *et al.* (2008) who showed that using KCl to replace sodium chloride promotes a less bitter and salty taste in food products.

Conclusion

We can conclude that starch had a desirable effect on sensory attributes; MTG and MTG associated with soy protein positively altered the texture. Partial replacement of sodium chloride by potassium chloride proved to be a feasible alternative in the preparation of restructured products based on non-commercial size trout fillets.

Acknowledgments

The authors are thankful for the financial support of the State of Rio de Janeiro Carlos Chagas Filho Research Foundation (FAPERJ), processes numbers E-26/103.003/2012 and E-26/110.406/2012. K.R. Palmeira and B.L. Rodrigues were supported by the National Council for Scientific and Technological Development (CNPq) and Coordination for the Improvement of Higher Education Personnel (CAPES).

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