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Aplicação da transglutaminase, resíduo de soja e substituição parcial do sal na elaboração de almôndega de Truta Arco-Íris (*Oncorhynchus mykiss*)

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Dissertação apresentada ao Programa de Pós-Graduação em Medicina Veterinária da Universidade Federal Fluminense, como requisito parcial para obtenção do grau de Mestre. Área de Concentração: Higiene Veterinária e Processamento Tecnológico de Produtos de Origem Animal.

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“Cada homem é uma casa espiritual que deve estar, por deliberação e esforço do morador, em contínua modificação para melhor.”

Emmanuel.

RESUMO

O consumo de pescado apesar de ser baixo no Brasil apresenta potencial de crescimento. O desenvolvimento de novos produtos utilizando esta matriz apresenta-se como uma alternativa para impulsionar a diversidade e distribuições de novos nichos de mercado. Devido à heterogeneidade de crescimento dos peixes, pode ocorrer o descarte daqueles que não atingem o tamanho comercial adequado, comprometendo a aceitação dos filés, que normalmente são subutilizados como resíduos de produção. Esta dissertação teve como objetivo elaborar um reestruturado, tipo almôndegas, utilizando-se resíduo do processamento de Truta arco-íris, proteína texturizada de soja (PTS), transglutaminase (TGM) e substituição parcial do cloreto de sódio por cloreto de potássio. Para o preparo do reestruturado, foram utilizados cinco tratamentos. O controle (T1) utilizando somente amido (4%), com 1% de transglutaminase (T2), com 4% PTS (T3) adicionado de 4% de PTS e 1% de TGM (T4) e utilizando 4% de PTS, 1% de TGM e substituição do cloreto de sódio por cloreto de potássio em proporção de 75%: 25% (T5). A elaboração de produtos derivados de pescado que apresentem valor agregado é um interesse para a indústria do setor e para os órgãos governamentais que visam o aumento no consumo destes produtos que apresentam características nutricionais desejáveis. Entretanto, dentre os produtos de origem animal, o pescado é o mais susceptível ao processo de deterioração, fato que destaca a importância de parâmetros e ferramentas analíticas que determinem seu estado de conservação. O presente estudo foi dividido em duas partes: (1) Avaliar a composição centesimal e a característica sensorial de almôndegas preparadas a partir de resíduos do processamento da Truta arco-íris (Artigo I); os resultados mostraram que a adição da transglutaminase e da proteína de soja, além da substituição parcial do cloreto de sódio pode ser alcançada sem prejudicar a característica físico-química e sensorial do produto. (2) Avaliar, através de parâmetros físico-químicos e bacteriológicos, os produtos elaborados com o resíduo de truta arco-íris (Artigo II); os resultados sugerem que a utilização combinada dos componentes associados ao armazenamento congelado pode ser aplicada como uma alternativa para a elaboração de novos produtos fabricados a partir de resíduos de truta. Baseado nos dados obtidos nas duas etapas do trabalho concluiu-se que a utilização dos resíduos pode ser uma alternativa viável como matéria-prima de baixo custo para a produção de novos produtos, diminuindo a poluição ambiental, agregando valor aos resíduos, possibilitando alternativa de lucro para indústria, além de atender a demanda dos consumidores por um alimento de fácil preparo, baixo custo e alto valor nutritivo.

Palavras-chave: transglutaminase, redução de sódio, truta arco-íris

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1 INTRODUÇÃO

O desenvolvimento econômico do país, associado às mudanças no hábito alimentar da população tem sido responsável pela crescente demanda por pescado no Brasil e no mundo. Apesar de as principais empresas brasileiras de proteínas não demonstrarem interesse por pescado, essa é a proteína de maior produção e consumo mundiais. A produção global de pescado foi de aproximadamente 148 milhões de toneladas em 2010, de acordo com a FAO. No Brasil, a produção total foi de 1.241 toneladas, conforme dados divulgados pelo Ministério da Pesca e Aquicultura (MPA) para 2009.

A média de consumo de pescado no mundo por ano é superior a 18 kg por habitante (FAO, 2011), no Brasil, a média de consumo per capita foi inferior, ficando próximo a 11,7 kg, dentro dos valores estabelecidos pela Organização Mundial da Saúde (OMS), que recomenda um consumo de 12 kg/hab/ano. No entanto, o consumo brasileiro cresceu 23,7% nos últimos 2 anos (MPA, 2013). A demanda mundial por pescado vem crescendo de forma acelerada em decorrência do aumento populacional e da busca por alimentos mais saudáveis.

Em 2009, o Brasil foi o 19º maior produtor de pescado do mundo, com 1.264.765 toneladas (0,75% do total), do total produzido pela piscicultura nacional (MPA, 2011), uma grande parcela é atribuída ao cultivo de carpas, trutas e tilápias (CREPALDI, 2006). Devido às suas características, sabor delicado e excelente qualidade nutricional, a Truta arco-íris (*Onchorynchus mykiss*) despertou grande interesse por parte de criadores em realizar o seu cultivo comercial (MACHADO et al., 2007). Até 2007, a produção nacional de trutas encontrava-se na faixa de 2500 toneladas por ano, o que correspondia a menos de 10% do volume de salmonídeos consumidos no Brasil, comercializada principalmente nas formas *in natura* e congelada, com peso ao redor de 300 gramas (AMARAL, 2007). Segundo o Boletim Estatístico da Pesca e Aquicultura, do Ministério da Pesca e Aquicultura, em 2009 a produção de trutas no Brasil foi superior a 4 mil toneladas por ano (BRASIL, 2009). A maioria das truticulturas brasileiras caracteriza-se como empreendimentos agrícolas do tipo familiar, pois os recursos hídricos favoráveis para a criação da truta são pouco volumosos, resultando em unidades com baixa escala de produção. A sustentabilidade econômica nessa atividade depende da utilização de tecnologias que proporcionem aumento da produtividade e diversificação de produtos de valor agregado (TABATA; PORTZ, 2004).

No sentido de promover o consumo do pescado, é preciso levar em consideração os desejos e preocupações do consumidor. Sendo assim, os produtos industrializados, oriundos da aquicultura, têm um grande mercado para ser explorado no Brasil, a exemplo do que ocorre em vários países, onde a diversidade de produtos industrializados está mais consolidado. O processamento e a industrialização permitem não só agregar valor, como também contribuir para a popularização do consumo do produto, como ocorreu na cadeia produtiva do frango, cuja expansão e a consolidação da atividade só se deram após uma mudança significativa nas formas de apresentação dos produtos (BARTOLOMEU, 2011).

2. FUNDAMENTAÇÃO TEÓRICA

2.1 RESÍDUOS

A sociedade tem voltado cada vez mais sua atenção para questões ambientais. Existe uma tendência mundial dos consumidores não se preocuparem apenas com a qualidade dos produtos, mas também com a responsabilidade com que os fabricantes demonstram na elaboração dos seus produtos, alguns consumidores pagam mais por produtos de empresas que não causam danos ao meio ambiente (SOBRAL, 2004). A gestão ambiental se enquadra justamente neste novo conceito da sociedade mundial em que as empresas passam a buscar uma melhoria contínua de seus produtos, no que se refere à qualidade, ao desenvolvimento sustentável e a redução de impactos negativos, para terem uma imagem positiva, junto aos consumidores de seus produtos (SPILLERE; BEAUMORD, 2006).

Segundo Silva e Maciel (2011), o termo resíduo sólido pode ser entendido como parte dos resíduos gerados que ainda possui valor comercial se for manejado adequadamente, ou seja, só depois de esgotar as possibilidades de utilizar o resíduo de uma atividade para outra é que se pode classificar esse material como descarte. A coleta e o tratamento de resíduos gerados pela perda de alimentos têm alto custo financeiro, social e ambiental. Os resíduos podem apresentar valor potencial, em particular os orgânicos, por sua capacidade de agregar valores econômicos e sociais ao seu tratamento, como o reaproveitamento destes resíduos e a reciclagem (PRIM, 2003).

A rejeição da matéria prima pode estar associada a diferentes fatores, tais como, perda por peso, perda da qualidade, o que acarreta rejeição tanto do varejista quanto do consumidor no momento da compra e perda de valor nutricional do produto devido a técnicas inadequadas de armazenamento ou manuseio incorreto, reduzindo a disponibilidade do alimento para o consumo *in natura* (CHITARRA; CHITARRA, 2005).

De acordo com Stevanato et al, (2007) o destino de 68% dos resíduos produzidos, por empresas do Sul do Brasil, são encaminhados às indústrias de farinha de pescado, 23% são encaminhados ao aterro sanitário municipal e 9% são despejados diretamente nos rios, constituindo assim um grave impacto ambiental. A média de descarte do pescado capturado, mesmo antes de chegar a indústria para beneficiamento é de aproximadamente 40% nas regiões Sul e Sudeste, ou seja, o pescado é rejeitado ainda na embarcação, podendo gerar potenciais fontes poluidoras de recursos hídricos, do solo e do ar (PESSATTI, 2001).

O aproveitamento de resíduos é de grande importância para evitar os desperdícios, reduzir os custos de produção do pescado e a poluição ambiental (STEVANATO et al., 2007). Segundo o mesmo autor, a criação de alternativas tecnológicas, com valor agregado que permitam o gerenciamento dos resíduos de pescado, podem trazer como resultado o combate à fome, a geração de empregos e o desenvolvimento sustentável.

As descobertas dos inúmeros danos ambientais, resultantes das práticas inadequadas de descarte de resíduo, têm aumentado o conhecimento e a preocupação dos consumidores sobre esta questão (MAZZER; CAVALCANTI, 2004). Nos últimos anos, esta preocupação tem sido manifestada e concretizada, através da elaboração e aprovação de leis federais, estaduais e municipais. Ainda segundo esses autores, paralelamente a conscientização do consumidor, vem o aumento da procura e aquisição de produtos que sejam considerados “verdes/limpos”, “ambientalmente corretos”, ou produtos que, além de apresentarem boa qualidade, possuam uma linha de produção que não provoque comprometimento ambiental. Esses aspectos vêm incentivando as indústrias a procurarem sistemas de produção eficazes que reduzam seus impactos ambientais, com custo de mercado compatível (MACÊDO, 2000).

As empresas estão adotando o Sistema de Gestão Ambiental (SGA), que as permite controlar permanentemente os efeitos ambientais de todo o seu processo de produção, desde a escolha da matéria-prima até o destino final do produto e dos resíduos líquidos, sólidos e gasosos, levando a empresa a funcionar de forma mais sustentável (BOUÇAS et al., 2009). Neste contexto, a certificação voluntária tem sido um instrumento capaz de promover a credibilidade das empresas frente ao comprometimento ambiental (MAZZER; CAVALCANTI, 2004), tornando assim o mercado mais competitivo tanto em âmbito nacional quanto em internacional.

2.2 ELABORAÇÃO DE NOVOS PRODUTOS À BASE DE PESCADO

O desenvolvimento de novos produtos é uma atividade de vital importância para a sobrevivência das indústrias, utilizando novas tecnologias e matérias-primas que seriam descartadas e apresentam potencial capacidade para serem industrializados (MACARI, 2007). Os resíduos gerados nas unidades processadoras e o pescado que apresenta baixo valor comercial e são descartados, acabam muitas vezes se tornando um sério problema ambiental (AMANCIO et al., 2010). Esses resíduos são largamente utilizados devido à facilidade de serem transformados em diversos produtos, e por apresentarem nutrientes de elevado valor biológico (STORI et al., 2002). O aproveitamento dos resíduos de pescado para consumo humano atende a demanda pelo aumento de consumo de ácidos graxos da série ômega-3, levando-se em consideração a importância nutricional na prevenção e no combate de doenças cardiovasculares, câncer e distúrbios neurológicos (MANSONA et al., 2012; DE CATERINA, 2011).

Em função das demandas por produtos com maior conveniência de preparo, muitas indústrias têm mostrado interesse em desenvolver novos produtos à base de pescado, que além de agregar valor, possibilitam o aumento do consumo deste alimento nobre e saudável (VEIT et al., 2011). Como o consumidor busca cada vez mais praticidade e alimentos de fácil manuseio, há empresas que aproveitam os resíduos do pescado para processá-los, transformando-os em nuggets, hambúrguer, almôndegas, empanados, entre outros produtos (SILVA; FERNANDES, 2010). O aproveitamento integral do pescado gera novos produtos e maior valor agregado. Como as carnes de frango, suína e bovina, o peixe pode ser inteiramente utilizado, agregando valor ao que seria descartado. Escamas e sangue podem ser aproveitados para produzir farinha e óleo de peixe (STEVANATO et al., 2007). Entretanto, para que seja possível o aproveitamento de todo o potencial de subprodutos, é necessário que haja viabilidade financeira.

A transformação destes resíduos em produtos para alimentação humana é uma ótima opção de renda para as indústrias, podendo aumentar sua lucratividade. Vidal et al. (2011) afirmam que a grande inovação da tecnologia para recuperação dos resíduos de pescado foi o aparecimento de equipamentos capazes de separar o material muscular agregado as espinhas com facilidade. A aceitação desses alimentos tem sido crescente uma vez que apresentam aparência, odor e sabor muito apreciados. Além disso, permitem agregar valor e conveniência, atendendo, dessa forma, interesses tanto dos frigoríficos, quanto dos consumidores e apresentam ainda maior prazo para consumo quando comparado ao pescado *in natura* (VEIT, 2011).

A elaboração de produtos industrializados derivados de pescado no Brasil é uma exigência permanente, pois estes se apresentam para a população como uma alternativa de consumo de produtos com elevado valor nutricional e preço acessível. Aliado a isto os consumidores terão acesso a produtos diferenciados, que podem apresentar-se em diferentes formas e texturas (SILVA, 2006).

Uma característica positiva na elaboração dos produtos derivados é que pode ser utilizado pescado de diferentes tamanhos, além destes produtos poderem ser adicionados de temperos, apresentando sabor e textura diferenciados sem haver rejeição dos consumidores (PEIXOTO et al., 2005). Existem várias técnicas de beneficiamento do pescado, sendo uma delas a utilização do pescado em hambúrgueres, almôndegas e nuggets, onde pode ser agregado valor nutricional ainda maior, através da adição de outros ingredientes como é a farinha de trigo, de soja e amido, além de atuar como ligantes devido a sua capacidade de reter água ainda atuam como agente texturizante (CORREIA, 2001).

Deve ser levado em consideração que parte da população não tem costume de se alimentar do pescado *in natura,* este desafio do baixo consumo pode ser solucionado com a elaboração de novos produtos, cuja diversificação das formas de processamento, pode oferecer ao pescado um aspecto mais aceitável pela população (SEBBEN, 2000).

Para elaboração destes produtos, as indústrias vêm utilizando ligantes que além das suas funções de emulsificação, absorção de água e gordura, e texturizantes, funcionam ainda como alimentos funcionais, como é o caso da proteína de soja. A soja pode ser considerada um alimento funcional, pois contém substâncias fisiologicamente ativas (MORAES, 2007) capazes de atuar como moduladores dos processos metabólicos, melhoria nas condições de saúde e do bem- estar e prevenção no surgimento de doenças degenerativas.

Outro ligante largamente utilizado é a transglutaminase, devido a sua grande habilidade de formar géis em soluções proteicas, a sua aplicação tem sido estudada em diversos alimentos (GERRARD, 2002). Esta enzima tem sido utilizada para modificar a funcionalidade de várias proteínas, incluindo as presentes em soja, miosina, glúten, caseínas e proteínas do soro (TRUONG et al., 2004). A habilidade da transglutaminase de modificar as propriedades funcionais dos alimentos proteicos tem sido uma das mais inovadoras utilizações de enzimas em tecnologia de alimentos da última década (LORENZEN, 2007).

Na elaboração dos novos produtos, para que seja atendido aos consumidores cada vez mais preocupados com a saúde, tem-se optado pela elaboração de produtos com substituição parcial do cloreto de sódio (SOARES et al., 2012). O grande desafio da indústria atualmente é o desenvolvimento de produtos que satisfaçam sensorialmente a expectativa dos consumidores e que ao mesmo tempo, possam ser consumidos sem causar danos a saúde. A reformulação de produtos através da substituição de ingredientes, como por exemplo, gordura e sal (NaCl), é uma alternativa para reduzir a associação que os consumidores fazem entre o consumo de produtos cárneos e problemas como a obesidade (BARRETTO; POLLONIO, 2007). Neste contexto pesquisadores tem direcionado os estudos para utilização de substitutos de sal em produtos cárneos como, por exemplo, o cloreto de potássio (KCl) e o cloreto de magnésio (Mg_2 Cl) (ALIÑO et al., 2010). O KCl possui propriedades similares ao NaCl e é reconhecido como seguro (GRAS), podendo ser usado na sua substituição sem perda da funcionalidade (VOGEL et al., 2011). Entretanto, o KCl possui sabor amargo, fato que provoca uma restrição em relação a adição deste em produtos cárneos, sendo a concentração de 1% considerada como o limite máximo de utilização (NASCIMENTO et al., 2008).

2.3 MÉTODOS ANALÍTICOS

2.3.1 **Análises bacteriológicas**

Alimentos podem ser contaminados durante manipulação e processamento. Após a contaminação o alimento serve como meio para o crescimento de microrganismos, podendo alterar as características físicas, químicas e sensoriais do alimento levando o mesmo a deterioração, tornando a análise bacteriológica importante, pois inúmeros métodos laboratoriais podem ser utilizados para determinar a presença destes microrganismos (FRANCO; LANDGRAF, 2003).

A contagem total de microrganismos aeróbios mesófilos quantifica as bactérias aeróbias e/ou facultativas e mesófilas (35 - 37°C), presentes tanto sob a forma vegetativa quanto esporulada (CUNHA, 2009). Segundo o mesmo autor, o número de microrganismo aeróbios e mesófilos (contagem em placa) encontrado em um alimento tem sido um dos indicadores de qualidade dos alimentos mais utilizados, indicando se a limpeza, a desinfecção e o controle da temperatura durante o processamento dos produtos, além do transporte e armazenamento, foram realizados de forma adequada.

2.3.2 Análises físico – químicas

As análises físico-químicas são realizadas com o objetivo de avaliar a qualidade dos alimentos. A importância das análises consiste na identificação das alterações físico-químicas a partir de compostos oriundos do processo de degradação, e outras alterações que interferem na qualidade do produto, proporcionando risco à saúde dos consumidores (ZOCCHE et al., 2002).

2.3.2.1 Composição centesimal

A Composição Centesimal de um alimento consiste na quantificação de determinadas substâncias em 100g do produto. As análises realizadas estão de acordo com as normas analíticas da Association of Official Analytical Chemists (AOAC, 2005). Convencionalmente, os grupos homogêneos de substâncias determinadas nessa análise são:

- Umidade: foi realizada de acordo com o método de secagem em estufa a 105ºC, onde a água é retirada por ação do calor e o teor de umidade é calculado pela diferença de peso das amostras no início e final do processo, quando atingem peso constante;

- Cinzas ou resíduo mineral fixo: foi obtido de acordo com o método de incineração em mufla, após a carbonização da matéria orgânica em bico de bunsen;

- Lipídios, gorduras ou extrato etéreo: foi realizada de acordo com o método de Soxhlet, utilizando o éter de petróleo como solvente;

- Proteínas: foi realizada de acordo com o método de micro Kjeldhal, que consiste nas etapas de digestão,destilação e titulação;

- Carboidratos: foi quantificado pelo método do Ácido Dinitrosalicilico (DNS) descrito por Miller (1959).

Podemos analisar assim a complexidade do alimento e o seu valor nutritivo (CARVALHO et al., 2002)

2.3.2.2 pH

As alterações do pH no pescado ocorrem pela ação das bactérias e atividade enzimática que alteram a concentração de hidrogênios livres e provocam a decomposição das moléculas (OGAWA; MAIA, 1999). Durante o rigor, o pH é variável, geralmente ficando próximo à neutralidade, acelerando a ação das enzimas musculares e das bactérias. Em seguida a fase de rigor, instala-se a fase de post rigor onde a actomiosina é degradada por enzimas proteolíticas, como a catepsina. Neste momento, ocorre a hidrólise proteica, formação de peptídeos e aminoácidos livres, que permitem a rápida ação dos microrganismos endógenos e exógenos, dando origem a substâncias nitrogenadas voláteis, ocasionando o aumento do pH (PACHECO et al, 2005).

2.3.2.3 Substâncias reativas ao Ácido Tiobarbitúrico (TBARS)

Os lipídios desempenham um importante papel na qualidade dos alimentos, particularmente em relação às propriedades sensoriais. Conferem também valor nutritivo aos alimentos, constituindo uma fonte de energia, de ácidos graxos essenciais (ácidos linoleico e linolênico) e de vitaminas lipossolúveis (A, D, E e K). A oxidação lipídica é um fenômeno espontâneo e inevitável e está diretamente ligada ao desenvolvimento do ranço oxidativo. Esta reação diminui o valor nutritivo dos alimentos e pode gerar compostos nocivos (SILVA et al., 1999).

O teste do número de substâncias reativas ao Ácido 2-Tiobarbitúrico (TBA) é baseado na reação do ácido tiobarbitúrico com os produtos de decomposição dos hidroperóxidos. Um dos principais produtos formados no processo oxidativo é o malonaldeído (MA), uma molécula de MA reage com duas moléculas de TBA para formar um complexo de cor rosada e sua leitura é feito por espectrofotômetro (OSAWA et al., 2005).

2.3.2.4 Aminas Biogênicas

As aminas biogênicas são compostos orgânicos nitrogenados, formados principalmente por descarboxilação de aminoácidos e estão presentes em alimentos como frutas e verduras, carne, peixe, chocolate e leite (SILVA et al., 2013). Estas podem acumular-se em concentrações elevadas e provocar consequências toxicológicas, sendo assim, a determinação da concentração das aminas biogênicas em um determinado alimento é importante devido à sua capacidade de ter um efeito direto ou indireto no sistema vascular e nervoso nos humanos (INNOCENTE et al., 2007).

As aminas são resistentes aos tratamentos térmicos aplicados no processamento de alimentos e têm sido consideradas adequadas indicadoras de frescor e decomposição de alimentos, refletindo a qualidade da matéria-prima e as condições higiênicas durante a produção (ÖZOGUL et al., 2002, GLÓRIA, 2006).

Segundo Bunková et al.(2010), a presença das aminas ocorre em alimentos protéicos ou que contenham aminoácidos livres ou seus precursores, especialmente em alimentos que forneçam condições ideais para atividade bioquímica dos microrganismos presentes. Ainda segundo o mesmo autor, além da disponibilidade de precursores (aminoácidos), a produção de aminas biogênicas depende de fatores extrínsecos e intrínsecos do alimento como temperatura, pH do meio, disponibilidade de fontes de carbono, entre outros, sendo o tipo de amina produzida, dependente da presença de aminoácidos precursores.

As aminas biogênicas mais encontradas no pescado podem ser classificadas em monoaminas aromáticas (tiramina), aminas heterocíclicas (histamina, triptamina), diaminas alifáticas (putrescina, cadaverina), e poliaminas alifáticas (agmatina, espermina, espermidina), esta classificação é dada devido a sua estrutura química (VIDAL-CAROU et al., 2009). A maioria das aminas foi nomeada a partir de seus aminoácidos precursores, porém alguns nomes como cadaverina e putrescina estão associados à decomposição e à putrefação, e espermina e espermidina se relacionam ao fluido seminal, do qual foram isoladas pela primeira vez (GLÓRIA, 2006).

A histamina tem sido associada à diversos surtos de intoxicação alimentar, principalmente pelo consumo de peixes, enquanto a tiramina tem sido associada à crises hipertensivas (COÏSSON et al., 2004). Segundo Koutsoumanis et al. (2010) outras aminas como cadaverina, putrescina e a tiramina, parecem aumentar a toxicidade de histamina. Além disso, as aminas biogênicas são consideradas como potencialmente carcinogênicas, pois têm a habilidade de reagir com nitratos e formar nitrosaminas carcinogênicas (KOUTSOUMANIS et al., 2010).

A sensibilidade humana em relação as aminas biogênicas varia de acordo com as atividades de detoxificação individual das enzimas envolvidas especificamente no metabolismo deste compostos, pois estas enzimas são inibidas por vários tipos de drogas, como antidepressivos e medicação anti- Parkinson (YOUDIM; WEINSTOCK, 2004).

Dentre as aminas biogênicas, apenas a concentração de histamina já foi regulamentada no Brasil. O governo norte-americano fixou um limite de 5 mg de histamina/100g de atum e peixes suscetíveis a formação das aminas (RIGUEIRA, 2009), a União Européia estabelece limites de histamina em alguns peixes marinhos utilizando um plano de três classes que determina que em um lote de nove amostras, nenhuma pode exceder 200mg/kg de histamina (M); e não mais que 2 amostras podem conter mais que 100mg/kg (m) (RAUSCHER-GABERING et al.,2009). No Brasil, a concentração máxima de histamina permitida no músculo de espécies pertencentes às famílias Scombridae, Scombresocidae, Clupeidae, Coryphaenidae é de 100ppm (BRASIL, 1997).

2.3.2.4.1 Métodos de detecção e quantificação das aminas biogênicas

As aminas biogênicas são analisadas para controle de qualidade das matérias-primas, produtos intermediários e produtos finais, desenvolvimento de novos produtos, controle de processamento e armazenamento (SILVA et al., 2013). Diversos métodos são utilizados para a análise de aminas biogênicas em alimentos, dentre eles está a cromatografia em camada fina, cromatografia gasosa, método de eletroforese capilar e a cromatografia líquida de alta eficiência (ÖNAL, 2007). Todos os métodos utilizam pelo menos a etapa de extração das aminas e sua quantificação (VINCI; ANTONELLI, 2002).

A Cromatografia Líquida de Alta Performance (HPLC) é uma técnica confiável e de alta sensibilidade para detecção e quantificação de diferentes aminas biogênicas (EFSA, 2011). Nos últimos anos, novas técnicas utilizando o HPLC para detectar e quantificar as aminas biogênicas vem sendo desenvolvidas e validadas com frequência.

Em estudo recente, Cunha et al., (2012) detectaram e quantificaram as aminas biogênicas cadaverina, espermidina, histamina, putrescina e tiramina em amostras de queijos utilizando a técnica de cromatografia líquida de alta eficiência, com os seguintes parâmetros: limite de detecção, limite de quantificação e recuperação. A solução de 5% de ácido perclórico (HClO4) 1:1 (v:p) foi utilizada no processo de extração e derivatização das aminas com 40 µL de cloreto de benzoíla. Esta nova metodologia mostrou-se eficaz para realizar a detecção e a quantificação das aminas biogênicas nos alimentos analisados. Outra metodologia de HPLC foi validada por Lázaro et al., (2013) para determinar aminas biogênicas em carne de frango. Os autores utilizaram um sistema de eluição isocrática acoplado a um detector de UV (254 nm) para a identificação das aminas, após a realização da extração ácida e derivatização também com cloreto de benzoíla. Neste estudo, foram utilizados a seletividade, linearidade, precisão, recuperação, limite de detecção e quantificação e robustez como parâmetros de validação.

2.3.3 Análises sensoriais

A Associação Brasileira de Normas Técnicas define a análise sensorial como a disciplina científica usada para evocar, medir, analisar e interpretar reações das características dos alimentos e materiais como são percebidas pelos sentidos da visão, olfato, paladar, tato e audição (ABNT, 1993).

Segundo ABBAS et al. (2008), a avaliação sensorial sempre teve papel fundamental na análise da qualidade e frescor da indústria pesqueira. As diversas características sensoriais, tais como aparência, odor, coloração são ainda muito importantes nos sistemas de qualidade das indústrias processadoras, sendo a inspeção sensorial do pescado processado utilizada na indústria para identificar defeitos ocorridos durante manipulação e processamento.

No entanto, a análise sensorial tem sido considerada uma metodologia que apresenta vantagens como baixo custo, rapidez e relação direta com os padrões de aceitação do consumidor, pois exige a participação de um número representativo de julgadores treinados ou não para obtenção de resultados confiáveis e reprodutíveis (FURLAN, 2011). Segundo o mesmo autor, a análise sensorial baseia-se na avaliação dos atributos sensoriais considerados significativos, como aparência, textura, aroma, entre outros, por meio de um sistema de classificação por pontos de demérito por atributo. A soma dessas classificações quantifica a qualidade sensorial do produto classificando-o como próprio ou não para consumo humano (HUSS, 1995). O laboratório de análise sensorial deve conter: cabines individuais, para aplicação dos testes, devem ser limpas, livre de ruídos e odores e apresentar área com boa iluminação (FERREIRA et al., 2000).

2.3.3.1 Teste de aceitação e Escala do Ideal

O objetivo da avaliação sensorial é detectar diferenças entre os produtos baseado nas diferenças perceptíveis na intensidade de alguns atributos (FERREIRA et al., 2000). Segundo o mesmo autor, o teste de aceitação é uma importante ferramenta, pois utiliza diretamente a opinião do consumidor já estabelecido ou potencial de um produto, sobre características específicas do produto ou ideias sobre o mesmo. As principais aplicações dos testes de aceitação são a manutenção da qualidade do produto, otimização de produtos e/ou processos e desenvolvimento de novos produtos (BERGARA-ALMEIDA; SILVA, 2002).

A escala hedônica é usada para medir o nível de preferência de produtos alimentícios por uma população, relata os estados agradáveis e desagradáveis no organismo, mede o gostar ou desgostar de um alimento. A avaliação da escala hedônica é convertida em escores numéricos e analisados estatisticamente para determinar a diferença no grau de preferência entre amostras (LAND e SHEPHERD, 1988; ABNT, 1998).

Os provadores expressam a sua aceitação seguindo uma escala previamente estabelecida, que varia desde “gostar” até “desgostar” (CHAVES; SPROESSER, 2002). A escala hedônica estruturada em 9 pontos normalmente é utilizada para a realização do teste de aceitação (9 = gostei extremamente; 1 = desgostei extremamente) (STONE; SIDEL, 1993). Os testes de aceitação necessitam de um grande número de provadores não treinados (acima de 30) que representem a população de consumidores atuais ou potenciais do produto (CHAVES; SPROESSER, 2002).

2.3.3.2 Just-About-Right (JAR)

Com a intermitente busca das indústrias de alimentos por produtos ideais, sob o ponto de vista da análise sensorial, diferentes ferramentas podem ser utilizadas, através de um painel de consumidores, entre elas está a classificação de atributo ideal a partir da metodologia “Just-About-Right (JAR)” (CHAN et al., 2013).

A escala JAR é bipolar, com ambas as extremidades ancoradas pela descrição sensorial opostas e no meio ancorada como "praticamente certa", com a frase "just-about-right" que denota o mais aceitável (GACULA JR et al, 2007). No entanto, McBride (1985) argumentaram o oposto, isto é, JAR que pode ser interpretado como um ponto de referência para a intensidade em vez de aceitação.

O JAR é uma medida direta da percepção da intensidade dos atributos, mas esta análise não as quantifica diretamente. JAR é geralmente expressa como o percentual de entrevistados que consideram o nível de atributo como muito alto, muito baixo, e just-about-right. Embora existam muitas variações de escalas JAR, geralmente tais escalas consistem em cinco ou sete pontos (MEULLENET et al., 2007). A escala mais comumente utilizada nos testes sensoriais é a escala de 5 pontos na qual para cada atributo utiliza-se os seguintes parâmetros: demasiadamente pouco, pouco, ideal, muito, demasiadamente muito. Em alguns estudos, utilizou-se apenas as escalas "pouco”, "ideal" e "muito" para aperfeiçoar um atributo (CHAN et al., 2013). Além disso, os desvios do “Just About Right” podem ser relacionados com a aceitação global através da análise de penalidade (CAVITT et al., 2005), sendo os resultados expressos como percentagem de julgadores que consideraram os níveis dos atributos como ideal, abaixo ou acima do ideal (CHAMBERS; BAKER, 1996).

3 DESENVOLVIMENTO

3.1 Artigo 1: USE OF TRANSGLUTAMINASE, SOYBEAN WASTE AND SALT REPLACEMENT IN THE ELABORATION OF TROUT (*Oncorhynchus mykiss*) MEATBALL. Accepted to International Food Research Journal

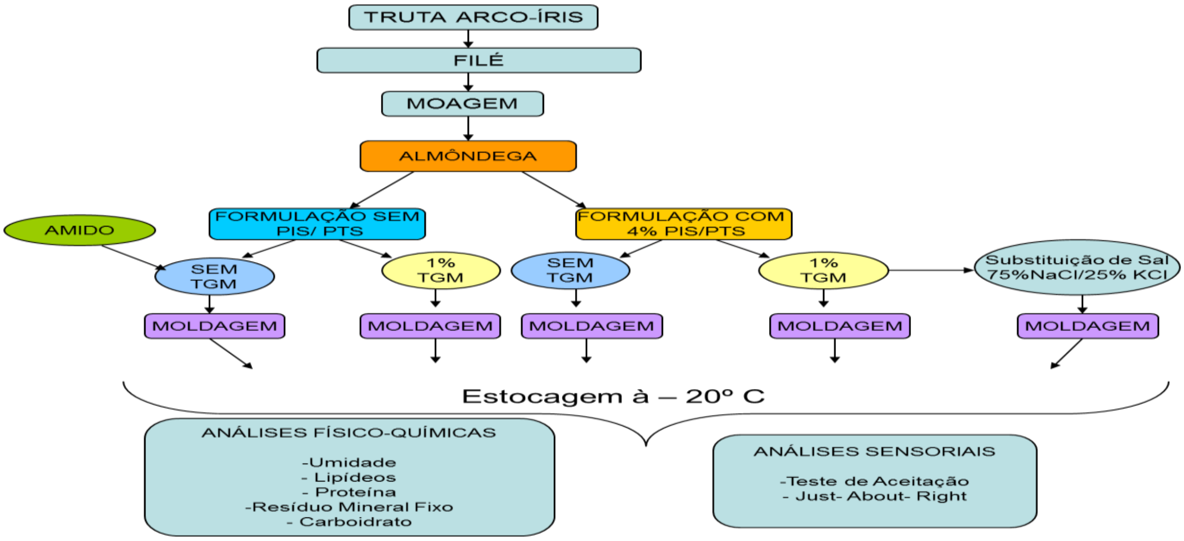

Figura 1. Desenho experimental delineado para desenvolvimento do artigo 1.

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

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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.

Keywords: Rainbow trout, centesimal composition, sensory analysis, penalty analysis.

Run title: Application of MTG and soybean in trout meatball

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 & 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).

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 & 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.

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

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 & 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 & 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.

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Table 1: Base formulation of the meatballs trout

Ingredients	T1	T2	T3	T4	T5
Fillets	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
Onion	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.

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.

**Different letters in the same line indicate significant differences ($P < 0.05$).

Table 3: Sensory attributes and meatballs acceptance.

Treatments/ Analyses	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.

** Different letters in the same line indicate significant differences ($P < 0.05$).

3.2 Artigo 2: QUALITY OF SEMI- PREPARED PRODUCTS FROM RAINBOW TROUT WASTE (*Onchorynchus mykiss*) BY USING DIFFERENT TECHNOLOGICAL STRATEGICS. Published in Food and Nutrition Science, v. 5, n. 6, p.571-580, 2014.
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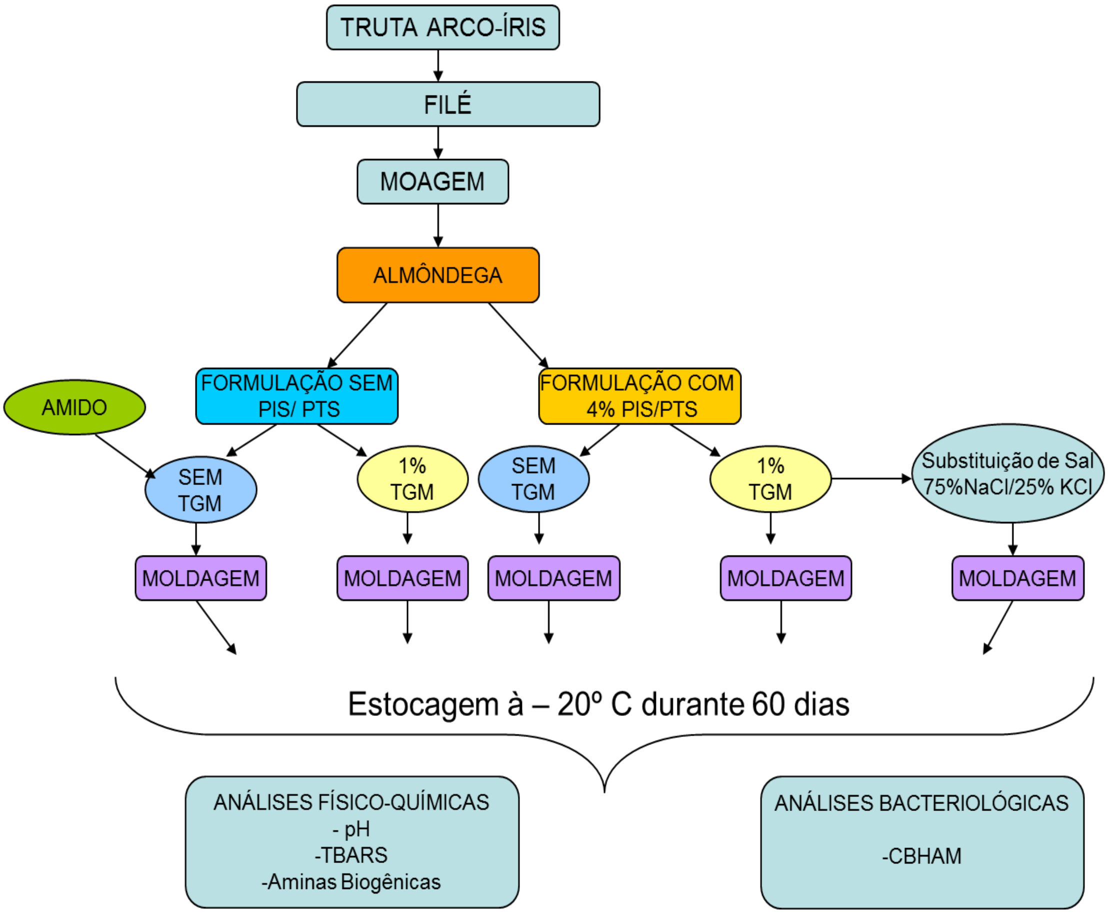

Figura 2. Desenho experimental delineado para desenvolvimento do artigo 2.

Quality of semi-prepared products from rainbow trout waste (*Onchorynchus mykiss*) by using different technological strategics

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Abstract

The consumption of freshwater fish and fish products has gradually grown worldwide over the last decades, generating a proportional waste increase. The objective of the present study was to assess the chemical and bacteriological quality of restructured fish product, meatball-type, prepared with rainbow trout (*Onchorynchus mykiss*) waste added of 1% transglutaminase (MTG), 4% textured soy protein (TSP) and replacing part of the sodium chloride with potassium chloride (75%/25%) as described below: 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). Total aerobic mesophilic bacteria (TAMB), 2-thiobarbituric acid reactive substances (TBARS), pH determination and quantification of biogenic amines were performed on the day after manufacturing (P0) and after 60 days of storage (P1) at -25±2^{o}C. The results showed that there was no significant difference ($p<0.05$) of microbiological quality, TBARS and pH after storage. T4 presented the lowest total biogenic amine content (256.84 mg/kg) where as T3 and T5 had the highest value (791.36 and 707.19 mg/kg, respectively) in this parameter. Putrescine was the biogenic amine that presented the highest concentration (504.00 mg/kg) in T3 and cadaverine the one that presented the smallest concentration (0.36 mg/kg) in T4. The use of technological strategies for developing new products with non-commercial fillets kept most standards, having changes only in some biogenic amines.

Keywords: *Oncorhynchus mykiss*, soy protein, microbial transglutaminase, salt replacer, biogenic amine.

1 Introduction

Freshwater fishes, such as rainbow trout are among the foods considered of high nutritional value, excellent source of proteins with high nutritional value and digestibility around 90%, besides calcium, unsaturated fatty acids (healthier) and vitamin B complex [1]. The average annual production of trout increased 18% in the last 10 years [2]. This growth was possible due to the increase in production scale and industrialization initiatives that favored trout marketing by large supermarket chains reaching markets in several metropolitan regions [3].

The fish industry, aiming at minimizing economic losses and environmental impact, has taken advantage of the less noble parts of certain food matrices to elaborate new products with added value [4]. The high production costs foster the study and the development of new technologies having in mind the use of all the animal parts to minimize waste and economic losses, and thus maximizing the companies' profit. This process includes the implementation of meat restructuring method on trimmings and low commercial value cuts to improve the appearance and texture of the new product, increasing its marketing value [5-6].

Restructuring processes are technologically very important because the products obtained have high commercial value, low production cost, are easy to use and marketing convenient. The use of transglutaminase (MTG) in the industrial preparation of these products has been widely studied, because its binding action does not depend on the use of sodium chloride or phosphates, generating healthier products because of lower levels of sodium salts [7]. Soy protein is an excellent source of essential amino acids, and has high digestibility, in addition some studies show that its consumption, is related to the reduction of non-infectious chronic diseases such as cardiovascular diseases, some types of cancer and osteoporosis [8]. However, due to high manipulation during the preparation of this product, the raw restructured must be kept frozen [9-10]. Although scholars are conducting research focusing on the development of new products, there is no work reported in the literature that addresses the addition of MTG and soy protein associated with partial replacement of sodium chloride in fish products.

Numerous bacteriological and physicochemical methods have been developed for quality assessment of fish and fish products, but the most used are TBAR, TVB, TMA, hypoxanthine, and pH determinations and microbiologic analysis [11], as well as the quantification of biogenic amines which, besides being of fundamental importance for human health, are taken into consideration as quality assessment criteria [12].

The objective of the present study was to evaluate the storage effect on the bacteriological and chemical quality of meatballs prepared with Rainbow Trout (*Oncorhynchus mykiss*) waste according to 5 different treatments using transglutaminase (MTG), textured soy protein and partial replacement of sodium chloride by potassium chloride and stored frozen at -25±2ºC for 60 days.

2. 2 Materials and methods

2.1 Preparation of Rainbow Trout Meatballs

The rainbow trouts (*Oncorhynchus mykiss*) were captured in tanks, and after removing the head were eviscerated and frozen. The restructured product was prepared with fillets of non-commercial size trouts which are treated as waste and were acquired at Morita Trout Farm, MG, Brazil. The base formulation was fillets, sugar, salt, garlic, onion, parsley and ascorbic acid. Five different treatments were made, with different contents of starch, soy protein and MTG, besides the partial replacement of sodium chloride by potassium chloride (Table 1). Starch was used when soy protein and MTG were not used. The frozen raw material was minced in a meat grinder using 3.0 mm disk and the ingredients and (dehydrated) seasonings were added in a technically important sequence (salt or NaCl/KCl mixture, starch or TSP and/or MTG, seasonings, sugar and finally ascorbic acid) and mixed until forming a homogenous mass (Table 1). The fish meatballs were manually shaped, weighing approximately 30 g each, packed in expanded polystyrene trays and stored at approximately -25±2°C. The bacteriological and chemical quality assessments were performed in duplicate on the 1st and 60th days of storage. Samples were randomly collected from each treatment from the frozen meatballs, stored at -25±2°C.

2.2 Bacteriological analysis

Media for the count of total aerobic mesophilic bacteria (TAMB) was Plate Count Agar (PCA) and the plates were inverted to incubation at 35±1ºC during 48 hours [13].

2.3. Chemical quality

The thiobarbituric acid reactive substances (TBARS) were determined by distilling the mixture of HCl and the sample added with distilled water, TBA was added to 25 mL of distilled and after immersion in a water bath and subsequent cooling, the reading was performed by the spectrophotometry (538 nm) described by [14] and adapted by [15]. The pH was determined in homogenized mixtures of meatballs and distilled water (1:10) [16], using a digital potentiometer (DIGIMED DM 22). Biogenic amines were quantified by High Performance Liquid Chromatography (HPLC) according to method described by [17]. The biogenic amines identified and quantified were: tyramine, putrescine, cadaverine, spermidine, histamine and spermine.

2.4. Statistical analysis

One-way ANOVA followed by the Tukey Test was performed to compare average of each parameter evaluated for each treatment between days of storage. Data were analyzed using GraphPad Prism 5 program® (GraphPad Software, San Diego, California, USA) at a 95% confidence level.

3.0 Results and discussion

3.1 **Bacteriological results**

There are no limits of TAMB in fish indicated by the brazilian legislation. However, the International Commission on Microbiological Specifications for Foods [18] recommends that the number of viable mesophilic bacteria does not exceed 10^6 CFU/g, because this count is considered critical in relation to the degree of product freshness. All the treatments presented counts below this limit and showed significant difference only in T1 after 60 days of storage, indicating that the meatballs were prepared under adequate sanitary conditions and that the storage maintained a controlled bacterial count. In the present study, all the values found in all the treatments were below 10^5 CFU/g, as shown in table 2. In agreement with our findings [19] reported counts values ranging beteween in a period of 60 days storage at – 18º in Rainbow Trout Kebab. [20] observed values below 10^5 CFU/g in fish burgers prepared with mechanically deboned meat (MDM) of Nile tilapia by using several binders and stored frozen. Through the bacteriological results, this study suggests the feasibility of using the rainbow trout in the preparation of meatballs

3.2 **Chemical quality results**

Table 3 summarizes the pH results of the 5 treatments which varied between 6.0 and 6.19 during the 60-day storage, but no significant difference ($p<0.05$) was observed.

According to our results, [21], observed a pH value of 6.55 in fish burger prepared with croaker waste. [22] studied Caranhas fish burger and found a mean pH value of 6.5 in fish burgers prepared with different salt contents. pH variation is related to storage conditions and the procedures to which the fish is submitted immediately after its capture, thus, this isolated value is not an exact criterion of meat freshness or quality, but must be interpreted in conjunction with other parameters [23-24].

An increase in the pH value in food matrices may suggest loss of quality, indicating accumulation of basic metabolites such as ammonia and some organic bases produced by microbial and endogenous enzyme actions [24-25]. [26] mentions the use of cold as a food preservation method, because it slows the action of spoilage agents and decreases the chemical reactions. According to [27], the pH value for fresh fish meat is 6.0-6.5, which can increase during storage because of accumulation of metabolites such as amines, indicating a spoilage process.

All the treatments showed TBARS values (mg MDA/kg) below the limits indicated in literature as sensory perceptible (between 5 and 8 mg MDA/kg) [28]. The values found in the first day and after 60-day storage, independent of treatment, suggest that the combination of the antioxidant (ascorbic acid) action and storage at -25±2ºC contributed to minimize the oxidation of the product. Another relevant factor is the average lipid content of this species of 4.95% described by [29], which classifies this species, according to [30] as lean fish (<5%), with consequent low percent degradation of the lipid molecule. According to [31] freezing drastically reduces the lipid oxidation process, because it reduces the rate of enzyme activity. This fact was also pointed out by [32] on their study on hamburgers prepared with mechanically deboned meat (MDM), stored raw and frozen (-20ºC). Besides, these authors noted that the treatment with ascorbic acid reduced the lipid oxidation rate.

The limits acknowledged by the scientific literature for TBARS were used, observing that values above 1-2 mg of malondialdehyde/kg fish are associated to rancid taste and smell [31]. Moreover, [32] report that TBARS values above 1.59 of malondialdehyde/kg sample can cause harm to consumer's health, since malonic dialdehyde and other products of lipid oxidation have been related to the onset of cancer and therefore have called the attention of the scientific community. On the other hand, [33] stated that a product with values below 3.0 of malondialdehyde/kg should be considered in good condition.

No difference ($p<0.05$) was observed among TBARS values (mg MDA/kg) during storage, except for T1 (control) which showed higher TBARS on the 60th day in comparasion to first day of storage. This fact may be attributed to presence of substances such as ketones, amino acids, oxidized proteins, carbohydrates, pyridines, esters, sugars. These compounds as well as malonaldehyde, can react with thiobarbituric acid (TBA) directly influencing the TBARS result, allowing to suggest that the TBARS significant increase only in the control samples, may have been caused by the presence of starch and not necessarily by degradation of the lipid fraction [34]. Nevertheless, although has been observed an increase of TBARS in T1 the values of the analyzed samples were within the limits indicated in literature.

Figure 1 shows the values of biogenic mines obtained by HPLC. The tyramine concentration found in the different treatments was not considered high in P0, with the lowest value in T5 (3.47 mg/kg) and the highest value in T1 (14.32 mg/kg). At the end of the 60-day storage (P1) tyramine concentration significantly ($p<0.05$) increased only in T1 and T2, where T2 reached the highest value (363.41 mg/kg). Tyramine is an important amine from the toxicological point of view because the detoxification mechanisms in humans may not be sufficient in case of high intake by allergic persons or patients that make use of drugs such as antidepressants and Parkinson disease symptom controllers [35]. The toxicity of tyramine and histamine are associated with the presence of alcohol and acetaldehyde, which can increase their toxic potential because they help to promote the transport of the biogenic amines to the intestinal wall [36]. According to [37] the toxic concentration of tyramine in healthy humans is 125 mg/kg, while in immunocompromised persons this value falls to 6 mg/Kg. Studies showed that in addition to its toxicity, tyramine promotes the adherence of pathogens such as *Escherichia coli* 0157:h7 on the gastric mucosa [38].

Histamine is considered the most important amine in fish [39] and the most toxic of the amines detected in food [40], but its toxic effects depend on concentration, the presence of other amines and the susceptibility of the individual, besides its health condition [41]. In the present study, the meatballs in treatments T1, T3 and T5 presented histamine levels of 106.15, 121.74 and 293.45 mg/kg, respectively, exceeding the maximum limit of 50 mg/kg established by the [42]. However, the samples of treatments T2 (23.24 mg/kg) and T4 (14.38 mg/kg) presented levels below the recommended limit and only the meatballs in treatment T5 exceeded the histamine limit established by the European Community [43]. Besides, T5 was the only treatment that presented a significant histamine content increase ($p<0.05$) after 60 days of storage (P1) when compared to the contents in P0. There are no official limits for other biogenic amines, but the concentration of amines such as tyramine should be observed when associated with histamine, putrescine and cadaverine to evaluate the overall presence of biogenic amines in the food matrices the intake of which can lead to alterations of the organism [36].

Although the count of heterotrophic aerobic mesophilic bacteria have been constant during the study may have occurred decreased counts of specific bacterial groups and the development of other genres. Accordingly, bacteria that produce decarboxylases enzymes may have been favored by selective pressure, including lactic acid bacteria *Tetragenococcus* *muriatics* [44-45-46] and *Citrobacter* spp beyond that was isolated in Rainbow Trout [47] and fish derivatives frozen[48-49]. Although there is no increase in the total bacterial load, the predominant bacterial groups of positive decarboxylase could explain the increase of histamine and tyramine during the storage.

Putrescine presented high values independent from treatment, with T5 showing the highest concentration (263, 27 mg/kg). When the samples were analyzed in P1, only T3 presented a significant increase ($p<0.05$). Arginine and proline are amino acids precursors of putrescine and are present in concentrations of 8 and 5.3% respectively in soy protein, a fact that can be associated with the significant increase of this biogenic amine in treatments with added soy protein [50].

On the other hand, cadaverine was the biogenic amine which kept the smallest levels both in P0 and P1. Cadaverine is mostly produced by spoilage bacteria that grow at high temperatures [51]. No significant difference was observed in any treatment after 60-day storage at -25±2ºC as well as on the mesophilic bacterial load was observed when P0 and P1 were compared (Figure 1). This behavior partially explains the absence of alteration of cadaverine levels. Despite the low levels found in the present study, cadaverine and putrescine are associated to the increase of histamine intestinal absorption, enhancing the toxicity of this amine, in addition to increasing the action of tyramine [52-53]. These amines can also act as nitrosamine precursors originating carcinogenic compounds [26].

Spermine was found in low concentrations in all the treatments. The levels of this amine presented reduction in all treatments. The behavior of spermidine was similar, with its highest value in T5 (141.69 mg/kg) in P0 and presenting significant reduction after the 60-day storage period (P1). According to [54], due to their endogenous origin, the limits of spermine and spermidine tend to decrease or remain the same, during storage, and for this reason these amines are generally not associated with health hazardous effects when evaluated separately [55], and are usually associated to allergic processes [3].

According to [41] the presence of biogenic amines in food is doubly important, first because the intake of products with high concentration of biogenic amines may cause significant toxicological and physiological effects to human health and second, because they may be used as quality indicators in certain foods. These authors also mention that the food industry is interested in reducing their levels in fish, because in addition to being linked to health and quality standards, they cause consumer rejection. They also state that biogenic amines can be used as quality control indices, because they vary during food preparation and storage, starting with low concentrations in the fresh product and increasing during storage associated to bacterial growth.

When the biogenic amine overall concentration was analyzed it was observed that the T3 samples after 60 days of storage and T5, independent of the storage time, presented the highest values. Soy protein addition in those treatments (T3 and T5) seems to have influenced the presence of these amines. According to [56], the amines present in higher concentration in soybeans are putrescine, cadaverine, agmatine, spermine and spermidine, because arginine, lysine, proline and tyrosine amino acids which are the precursors of the above mentioned biogenic amines are present in the composition of the soy protein.

In addition, in T5 there was replacement of 25% sodium chloride by potassium chloride. According to [57] some ingredients and additives have been used to increase the technological properties and / or sensory attributes during production or in the final product, however in some cases they can also act as inhibitors of bacterial growth, which is one of the conditions to form biogenic amines. The author also mentions that ingredients such as salt (NaCl) reduce the bacterial growth that interferes in the endogenous and exogenous proteolytic enzyme activity, inhibiting the formation of free amino acids and consequently reducing the formation of biogenic amines. In this manner, it seems that the replacement of 25% sodium chloride by potassium chloride may have influenced the overall amine values in T5.

Conclusions

The addition of transglutaminase (MTG), textured soy protein (TSP) and partial replacement of sodium chloride by potassium chloride in the product caused no significant changes during the period of storage in most parameters evaluated, only in some biogenic amines.

The biogenic amines proved to be a promising quality indicator in the studied products, mainly in those treatments with soy protein addition, and should be carefully evaluated in products prepared with fish waste and salt substitutes, because despite its health appeal, the technological effect of this ingredient may be compromised.

The present study suggests that the combined use of these ingredients associated to frozen storage can be applied as alternative for elaboration of new semi-prepared products manufactured from trout wastes leading advantages in the fish industry.

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Table 1: Base formulation of the meatballs trout

Ingredients	T1	T2	T3	T4	T5
Fillets	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
Onion	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).

Table 2: Count of aerobic mesophilic heterotrophic bacteria (UFC/g) of meatballs trout

Days of storage	T1	T2	T3	T4	T5
P0	$2.2x10^{4\ a}$	$2.5x10^{4\ a}$	$2.5x10^{4\ a}$	$5.3x10^{4\ a}$	$2.2x10^{4\ a}$
P1	$3.5x10^{2\ b}$	$1.4x10^{3\ a}$	$2.7x10^{3\ a}$	$1.4x10^{4\ a}$	$4.6x10^{3\ a}$

*UFC/g = colony forming units per gram of sample.

** 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).
***P0= day 1, P1= after 60 days of storage.
Different letters in the same column indicate significant differences ($P < 0.05$).

Table 3: Results of TBARS (mg MDA/kg) and pH analyzes of samples in different treatments of meatballs trout

Parameters	T1	T2	T3	T4	T5
TBARS (P0)	0.27[a] ± 0.01	0.12[a] ± 0.04	0.12[a] ± 0.01	0.12[a] ± 0.01	0.25[a] ± 0.08
TBARS (P1)	0.60[b] ± 0.08	0.23[a] ± 0.04	0.17[a] ± 0.03	0.17[a] ± 0.04	0.10[a] ± 0.02
pH (P0)	6.10 [a] ± 0.04	6.10 [a] ± 0.02	6.00 [a] ± 0.04	6.10 [a] ± 0.01	6.00 [a] ± 0.02
pH (P1)	6.17 [a] ± 0.01	6.15 [a] ± 0.01	6.08 [a] ± 0.03	6.17 [a] ± 0.04	6.19 [a] ± 0.01

* 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).
**TBARS (thiobarbituric acid reactive substances)
***P0= day 1, P1= after 60 days of storage.
Different letters in the same collum indicate significant differences ($P < 0.05$).

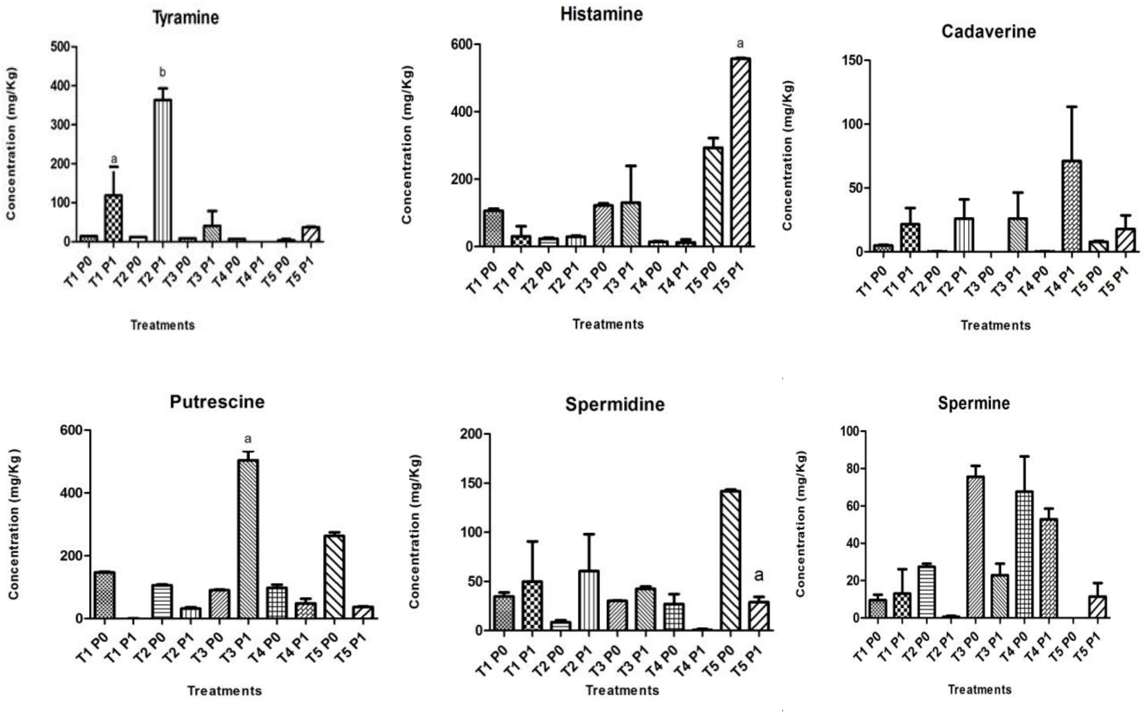

Figure 1: Amines in meatballs trout immediately after preparation (P0) and after 60 days of storage (P1).

*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 Values of Biogenic of salt).

**Different letters in the same graph indicate significant differences (P< 0.05)

4 CONSIDERAÇÕES FINAIS

A produção de novos produtos, utilizando resíduos oriundos do beneficiamento, pode ser uma alternativa viável, pois utiliza material de baixo custo ou baixo valor comercial, que seriam descartados, possibilitando assim a obtenção de lucro para a indústria. Além de evitar as perdas econômicas para as indústrias, diminui a poluição do ambiente, e possibilita uma alternativa de lucro, pois agrega valor aos resíduos e atende a busca dos consumidores por produtos sustentáveis, de baixo custo, fácil preparo e de elevado valor nutritivo.

A enzima transglutaminase e a proteína de soja mostraram-se capazes de manter as características físico-químicas do produto, sem influenciar na qualidade microbiológica durante os 60 dias de estocagem em congelamento. Estes ligantes apresentaram ainda a capacidade de melhorar atributos que contribuíram positivamente para a aceitação global, sendo a concentração de 0,5% de transglutaminase e 4% de proteína de soja suficientes para obtenção das almôndegas de Truta Arco-Íris apresentando elevada aceitabilidade. O cloreto de potássio (KCl) pode ser utilizado como substituto parcial do cloreto de sódio (NaCl), reduzindo os riscos a saúde quando associados ao elevado consumo de sódio. Baseado nos resultados obtidos, o produto elaborado demonstra-se como potencial alternativa para as indústrias de pescado.

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6 Apêndices

6.1 Aceite do artigo 1:

International Food Research Journal

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UPM/FSTM/A/SR/13/558

23rd January 2014

Dear Karoline Ribeiro Palmeira,

MANUSCRIPT IFRJ-2013-558

Your Manuscript entitled "**Use of transglutaminase, soybean waste and salt replacement in the elaboration of trout (*Oncorhynchus mykiss*) meatball**" has been accepted for publication in the International Food Research Journal. We thank you for your contribution to the International Food Research Journal and encourage you to submit other articles to the Journal.
Please fill and sign the attached forms and return to me to enable us to process your manuscript for publication.

Thank you.

Yours sincerely,

Professor Dr. Son Radu
Editor
International Food Research Journal
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6.2 Publicação do artigo 2

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Scientific Research

Quality of Semi-Prepared Products from Rainbow Trout Waste (*Onchorynchus mykiss*) by Using Different Technological Strategics

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Abstract

The consumption of freshwater fish and fish products has gradually grown worldwide over the last decades, generating a proportional waste increase. The objective of the present study was to assess the chemical and bacteriological quality of restructured fish product, meatball-type, prepared with rainbow trout (*Onchorynchus mykiss*) waste added of 1% transglutaminase (MTG), 4% textured soy protein (TSP) and replacing part of the sodium chloride with potassium chloride (75%/25%) as described below: 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). Total aerobic mesophilic bacteria (TAMB), 2-thiobarbituric acid reactive substances (TBARS), pH determination and quantification of biogenic amines were performed on the day after manufacturing (P0) and after 60 days of storage (P1) at −25˚C ± 2˚C. The results showed that there was no significant difference ($p < 0.05$) of microbiological quality, TBARS and pH after storage. T4 presented the lowest total biogenic amine content (256.84 mg/kg) whereas T3 and T5 had the highest value (791.36 and 707.19 mg/kg, respectively) in this parameter. Putrescine was the biogenic amine that presented the highest concentration (504.00 mg/kg) in T3 and cadaverine that presented the smallest concentration (0.36 mg/kg) in T4. The use of technological strategies for developing new products with non-commercial fillets kept the most standards, having changes only in some biogenic amines.

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Keywords

***Oncorhynchus mykiss*; Soy Protein; Microbial Transglutaminase; Salt Replacer; Biogenic Amine**

1. Introduction

Freshwater fishes, such as rainbow trout, are among the foods considered of high nutritional value, excellent source of proteins with high nutritional value and digestibility around 90%, besides calcium, unsaturated fatty acids (healthier) and vitamin B complex [1]. The average annual production of trout increased 18% in the last 10 years [2]. This growth was possible due to the increase in production scale and industrialization initiatives that favored trout marketing by large supermarket chains reaching markets in several metropolitan regions [3].

The fish industry, aiming at minimizing economic losses and environmental impact, has taken advantage of the less noble parts of certain food matrices to elaborate new products with added value [4]. The high production costs foster the study and the development of new technologies having in mind the use of all the animal parts to minimize waste and economic losses, and thus maximizing the companies' profit. This process includes the implementation of meat restructuring method on trimmings and low commercial value cuts to improve the appearance and texture of the new product, increasing its marketing value [5] [6].

Restructuring processes are technologically very important because the products obtained have high commercial value, low production cost, and are easy to use and marketing convenient. The use of transglutaminase (MTG) in the industrial preparation of these products has been widely studied, because its binding action does not depend on the use of sodium chloride or phosphates, generating healthier products because of lower levels of sodium salts [7]. Soy protein is an excellent source of essential amino acids, and has high digestibility, in addition some studies show that its consumption, is related to the reduction of non-infectious chronic diseases such as cardiovascular diseases, some types of cancer and osteoporosis [8]. However, due to high manipulation during the preparation of this product, the raw restructured must be kept frozen [9] [10]. Although scholars are conducting research focusing on the development of new products, there is no work reported in the literature that addresses the addition of MTG and soy protein associated with partial replacement of sodium chloride in fish products.

Numerous bacteriological and physicochemical methods have been developed for quality assessment of fish and fish products, but the most used are TBAR, TVB, TMA, hypoxanthine, and pH determinations and microbiologic analysis [11], as well as the quantification of biogenic amines which, besides being of fundamental imsportance for human health, are taken into consideration as quality assessment criteria [12].

The objective of the present study was to evaluate the storage effect on the bacteriological and chemical quality of meatballs prepared with Rainbow Trout (*Oncorhynchus mykiss*) waste according to 5 different treatments using transglutaminase (MTG), textured soy protein and partial replacement of sodium chloride by potassium chloride and stored frozen at −25˚C ± 2˚C for 60 days.

2 Materials and Methods

2.1. Preparation of Rainbow Trout Meatballs

The rainbow trouts (*Oncorhynchus mykiss*) were captured in tanks, and after removing the head were eviscerated and frozen. The restructured product was prepared with fillets of non-commercial size trouts which are treated as waste and were acquired at Morita Trout Farm, MG, Brazil. The base formulation was fillets, sugar, salt, garlic, onion, parsley and ascorbic acid. Five different treatments were made, with different contents of starch, soy protein and MTG, besides the partial replacement of sodium chloride by potassium chloride (**Table 1**). Starch was used when soy protein and MTG were not used. The frozen raw material was minced in a meat grinder using 3.0 mm disk and the ingredients and (dehydrated) seasonings were added in a technically important sequence (salt or NaCl/KCl mixture, starch or TSP and/or MTG, seasonings, sugar and finally ascorbic acid) and mixed until forming a homogenous mass (**Table 1**). The fish meatballs were manually shaped, weighing approximately 30 g each, packed in expanded polystyrene trays and stored at approximately −25˚C ± 2˚C. The

Table 1. Base formulation of the meatballs trout (g/100g).

Ingredients	T1	T2	T3	T4	T5
Fillets	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).

bacteriological and chemical quality assessments were performed in duplicate on the 1^{st} and 60^{th} days of storage. Samples were randomly collected from each treatment from the frozen meatballs, stored at −25˚C ± 2˚C.

2.2. Bacteriological Analysis

Mediafor the count of total aerobic mesophilic bacteria (TAMB) was Plate Count Agar (PCA) and the plates were inverted to incubation at 35˚C ± 1˚C during 48 hours [13].

2.3. Chemical Quality

The thiobarbituric acid reactive substances (TBARS) were determined by distilling the mixture of HCl and the sample added with distilled water, TBA was added to 25 mL of distilled and after immersion in a water bath and subsequent cooling, the reading was performed by the spectrophotometry (538 nm) described by [14] and adapted by [15]. The pH was determined in homogenized mixtures of meatballs and distilled water (1:10) [16], using a digital potentiometer (DIGIMED DM 22). Biogenic amines were quantified by High Performance Liquid Chromatography (HPLC) according to method described by [17]. The biogenic amines identified and quantified were: tyramine, putrescine, cadaverine, spermidine, histamine and spermine.

2.4. Statistical Analysis

One-way ANOVA followed by the Tukey Test was performed to compare average of each parameter evaluated for each treatment between days of storage. Data were analyzed using GraphPad Prism 5 program® (GraphPad Software, San Diego, California, USA) at a 95% confidence level.

3. Results and Discussion

3.1. Bacteriological Results

There are no limits of TAMB in fish indicated by the brazilian legislation. However, the International Commission on Microbiological Specifications for Foods [18] recommends that the number of viable mesophilic bacteria does not exceed 10^6 CFU/g, because this count is considered critical in relation to the degree of product freshness. All the treatments presented counts below this limit and showed significant difference only in T1 after 60 days of storage, indicating that the meatballs were prepared under adequate sanitary conditions and that the storage maintained a controlled bacterial count. In the present study, all the values found in all the treatments were below 10^5 CFU/g, as shown in Table 2. In agreement with our findings [19] reported counts values ranging beteween in a period of 60 days storage at −18˚ in Rainbow Trout Kebab. [20] observed values below 10^5 CFU/g in fish burgers prepared with mechanically deboned meat (MDM) of Nile tilapia by using several binders and stored frozen. Through the bacteriological results, this study suggests the feasibility of using the rainbow trout in the preparation of meatballs

3.2. Chemical Quality Results

Table 3 summarizes the pH results of the 5 treatments which varied between 6.0 and 6.19 during the 60-day storage, but no significant difference ($p < 0.05$) was observed.

According to our results [21], observed a pH value of 6.55 in fish burger prepared with croaker waste. [22] studied Caranhas fish burger and found a mean pH value of 6.5 in fish burgers prepared with different salt contents. pH variation is related to storage conditions and the procedures to which the fish is submitted immediately after its capture, thus, this isolated value is not an exact criterion of meat freshness or quality, but must be interpreted in conjunction with other parameters [23] [24].

An increase in the pH value in food matrices may suggest loss of quality, indicating accumulation of basic metabolites such as ammonia and some organic bases produced by microbial and endogenous enzyme actions [24]-[26] mentions the use of cold as a food preservation method, because it slows the action of spoilage agents and decreases the chemical reactions. According to [27], the pH value for fresh fish meat is 6.0 - 6.5, which can increase during storage because of accumulation of metabolites such as amines, indicating a spoilage process.

All the treatments showed TBARS values (mg MDA/kg) below the limits indicated in literature as sensory perceptible (between 5 and 8 mg MDA/kg) [28]. The values found in the first day and after 60-day storage, independent of treatment, suggest that the combination of the antioxidant (ascorbic acid) action and storage at −25˚C ± 2˚C contributed to minimize the oxidation of the product. Another relevant factor is the average lipid content of this species of 4.95% described by [29], which classifies this species, according to [30] as lean fish (<5%), with consequent low percent degradation of the lipid molecule. According to [31] freezing drastically reduces the lipid oxidation process, because it reduces the rate of enzyme activity. This fact was also pointed out by [32] on their study on hamburgers prepared with mechanically deboned meat (MDM), stored raw and frozen (−20˚C). Besides, these authors noted that the treatment with ascorbic acid reduced the lipid oxidation rate.

The limits acknowledged by the scientific literature for TBARS were used, observing that values above 1 - 2 mg of malondialdehyde/kg fish are associated to rancid taste and smell [31]. Moreover, [32] report that TBARS values above 1.59 of malondialdehyde/kg sample can cause harm to consumer's health, since malonic dialdehyde and other products of lipid oxidation have been related to the onset of cancer and therefore have called the attention of the scientific community. On the other hand, [33] stated that a product with values below 3.0 of malondialdehyde/kg should be considered in good condition.

Table 2. Count of aerobic mesophilic heterotrophic bacteria (UFC. g^{-1}) of meatballs trout.

Days of storage	T1	T2	T3	T4	T5
P0	2.2×10^{4a}	2.5×10^{4a}	2.5×10^{4a}	5.3×10^{4a}	2.2×10^{4a}
P1	3.5×10^{3a}	1.4×10^{4a}	2.7×10^{4a}	1.4×10^{4a}	4.6×10^{4a}

*UFC. g^{-1} − colony forming units per gram of sample. **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). ***P0 − day 1, P1 − after 60 days of storage. Different letters in the same column indicate significant differences ($P < 0.05$).

Table 3. Results of TBARS (mg MDA/kg) and pH analyses of samples in different treatments of meatballs trout.

Parameters	T1	T2	T3	T4	T5
TBARS (P0)	$0.27^a \pm 0.01$	$0.12^a \pm 0.04$	$0.12^a \pm 0.01$	$0.12^a \pm 0.01$	$0.25^a \pm 0.08$
TBARS (P1)	$0.60^b \pm 0.08$	$0.23^a \pm 0.04$	$0.17^a \pm 0.03$	$0.17^a \pm 0.04$	$0.10^a \pm 0.02$
pH (P0)	$6.10^a \pm 0.04$	$6.10^a \pm 0.02$	$6.00^a \pm 0.04$	$6.10^a \pm 0.01$	$6.00^a \pm 0.02$
pH (P1)	$6.17^a \pm 0.01$	$6.15^a \pm 0.01$	$6.08^a \pm 0.03$	$6.17^a \pm 0.04$	$6.19^a \pm 0.01$

*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). **TBARS (thiobarbituric acid reactive substances) ***P0 − day 1, P1 − after 60 days of storage. Different letters in the same line indicate significant differences ($P < 0.05$).

No difference (P < 0.05) was observed among TBARS values (mg MDA/kg) during storage, except for T1 (control) which showed higher TBARS on the 60th day in comparasion to first day of storage. This fact may be attributed to presence of substances such as ketones, amino acids, oxidized proteins, carbohydrates, pyridines, esters, sugars. These compounds as well as malonaldehyde, can react with thiobarbituric acid (TBA) directly influencing the TBARS result, allowing to suggest that the TBARS significant increase only in the control samples, may have been caused by the presence of starch and not necessarily by degradation of the lipid fraction [34]. Nevertheless, although has been observed an increase of TBARS in T1 the values of the analyzed samples were within the limits indicated in literature.

Figure 1 shows the values of biogenic mines obtained by HPLC. The tyramine concentration found in the different treatments was not considered high in P0, with the lowest value in T5 (3.47 mg/kg) and the highest value in T1 (14.32 mg/kg). At the end of the 60-day storage (P1) tyramine concentration significantly (P < 0.05) increased only in T1 and T2, where T2 reached the highest value (363.41 mg/kg). Tyramine is an important amine from the toxicological point of view because the detoxification mechanisms in humans may not be sufficient in case of high intake by allergic persons or patients that make use of drugs such as antidepressants and Parkinson disease symptom controllers [35]. The toxicity of tyramine and histamine are associated with the presence of alcohol and acetaldehyde, which can increase their toxic potential because they help to promote the transport of the biogenic amines to the intestinal wall [36]. According to [37] the toxic concentration of tyramine in healthy humans is 125 mg/kg, while in immunocompromised persons this value falls to 6 mg/Kg. Studies showed that in addition to its toxicity, tyramine promotes the adherence of pathogens such as *Escherichia coli* 0157:h7 on the gastric mucosa [38].

Histamine is considered the most important amine in fish [39] and the most toxic of the amines detected in food [40], but its toxic effects depend on concentration, the presence of other amines and the susceptibility of the individual, besides its health condition [41]. In the present study, the meatballs in treatments T1, T3 and T5 presented histamine levels of 106.15, 121.74 and 293.45 mg/kg, respectively, exceeding the maximum limit of 50 mg/kg established by the [42]. However, the samples of treatments T2 (23.24 mg/kg) and T4 (14.38 mg/kg) presented levels below the recommended limit and only the meatballs in treatment T5 exceeded the histamine limit established by the European Community [43]. Besides, T5 was the only treatment that presented a significant histamine content increase (P < 0.05) after 60 days of storage (P1) when compared to the contents in P0.

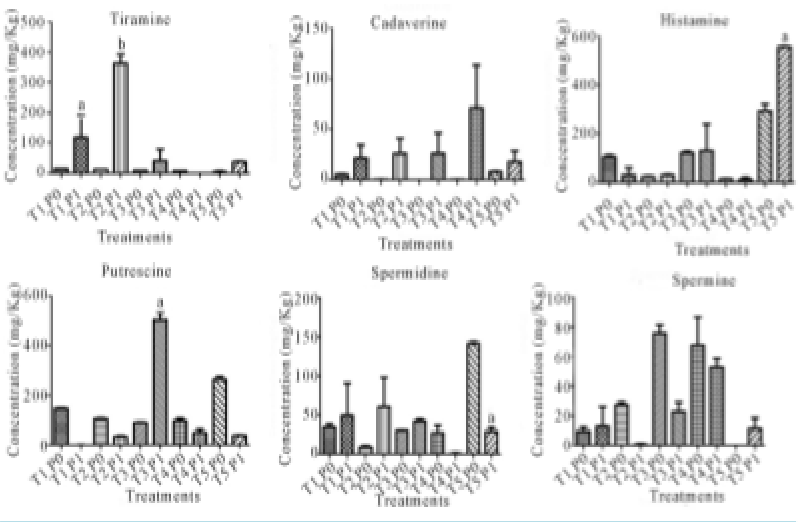

Figure 1. Values of biogenic amines in meatballs trout immediately after preparation (P0) and after 60 days of storage (P1). *T1 (starch addition), T2 (MTG addition), T3 (soy protein addition), T4 (soy protein and MTG addition) and T5 (soy protein addition, MTG addition and partial replacement of salt). Different letters in the same graph indicate significant differences (P < 0.05).

There are no official limits for other biogenic amines, but the concentration of amines such as tyramine should be observed when associated with histamine, putrescine and cadaverine to evaluate the overall presence of biogenic amines in the food matrices the intake of which can lead to alterations of the organism [36].

Although the count of heterotrophic aerobic mesophilic bacteria have been constant during the study may have occurred decreased counts of specific bacterial groups and the development of other genres. Accordingly, bacteria that produce decarboxylases enzymes may have been favored by selective pressure, including lactic acid bacteria *Tetragenococcus muriatics* [44]-[46] and *Citrobacter* spp beyond that was isolated in Rainbow Trout [47] and fish derivatives frozen [48] [49]. Although there is no increase in the total bacterial load, the predominant bacterial groups of positive decarboxylase could explain the increase of histamine and tyramine during the storage.

Putrescine presented high values independent from treatment, with T5 showing the highest concentration (263.27 mg/kg). When the samples were analyzed in P1, only T3 presented a significant increase ($P < 0.05$). Arginine and proline are amino acids precursors of putrescine and are present in concentrations of 8 and 5.3% respectively in soy protein, a fact that can be associated with the significant increase of this biogenic amine in treatments with added soy protein [50].

On the other hand, cadaverine was the biogenic amine which kept the smallest levels both in P0 and P1. Cadaverine is mostly produced by spoilage bacteria that grow at high temperatures [51]. No significant difference was observed in any treatment after 60-day storage at −25˚C ± 2˚C as well as on the mesophilic bacterial load was observed when P0 and P1 were compared (**Figure 1**). This behavior partially explains the absence of alteration of cadaverine levels. Despite the low levels found in the present study, cadaverine and putrescine are associated to the increase of histamine intestinal absorption, enhancing the toxicity of this amine, in addition to increasing the action of tyramine [52] [53]. These amines can also act as nitrosamine precursors originating carcinogenic compounds [26].

Spermine was found in low concentrations in all the treatments. The levels of this amine presented reduction in all treatments. The behavior of spermidine was similar, with its highest value in T5 (141.69 mg/kg) in P0 and presenting significant reduction after the 60-day storage period (P1). According to [54], due to their endogenous origin, the limits of spermine and spermidine tend to decrease or remain the same, during storage, and for this reason these amines are generally not associated with health hazardous effects when evaluated separately [55], and are usually associated to allergic processes [3].

According to [41] the presence of biogenic amines in food is doubly important, first because the intake of products with high concentration of biogenic amines may cause significant toxicological and physiological effects to human health and second, because they may be used as quality indicators in certain foods. These authors also mention that the food industry is interested in reducing their levels in fish, because in addition to being linked to health and quality standards, they cause consumer rejection. They also state that biogenic amines can be used as quality control indices, because they vary during food preparation and storage, starting with low concentrations in the fresh product and increasing during storage associated to bacterial growth.

When the biogenic amine overall concentration was analyzed it was observed that the T3 samples after 60 days of storage and T5, independent of the storage time, presented the highest values. Soy protein addition in those treatments (T3 and T5) seems to have influenced the presence of these amines. According to [56], the amines present in higher concentration in soybeans are putrescine, cadaverine, agmatine, spermine and spermidine, because arginine, lysine, proline and tyrosine amino acids which are the precursors of the above mentioned biogenic amines are present in the composition of the soy protein.

In addition, in T5 there was replacement of 25% sodium chloride by potassium chloride. According to [57] some ingredients and additives have been used to increase the technological properties and/or sensory attributes during production or in the final product, however in some cases they can also act as inhibitors of bacterial growth, which is one of the conditions to form biogenic amines. The author also mentions that ingredients such as salt (NaCl) reduce the bacterial growth that interferes in the endogenous and exogenous proteolytic enzyme activity, inhibiting the formation of free amino acids and consequently reducing the formation of biogenic amines. In this manner, it seems that the replacement of 25% sodium chloride by potassium chloride may have influenced the overall amine values in T5.

4. Conclusions

The addition of transglutaminase (MTG), textured soy protein (TSP) and partial replacement of sodium chloride by potassium chloride in the product caused no significant changes during the period of storage in most parameters evaluated, only in some biogenic amines.

The biogenic amines proved to be a promising quality indicator in the studied products, mainly in those treatments with soy protein addition, and should be carefully evaluated in products prepared with fish waste and salt substitutes, because despite its health appeal, the technological effect of this ingredient may be compromised.

The present study suggests that the combined use of these ingredients associated to frozen storage can be applied as alternative for elaboration of new semi-prepared products manufactured from trout wastes leading advantages in the fish industry.

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List of Abbreviations

CFU = colony forming units
g = gramme
HCl = hydrochloric acid
HPLC = High Performance Liquid Chromatography
KCl = potassium chloride
Kg = kilogram
MDA = malondialdehyde
MDM = mechanically deboned meat
MTG = transglutaminase
NaCl = sodium chloride
PCA = Plate Count Agar
TAMB = Total aerobic mesophilic bacteria
TBARS = 2-thiobarbituric acid reactive substances
TMA = trimethylamine
TSP = textured soy protein
TVB = total volatile bases