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EXTRAÇÃO, CARACTERIZAÇÃO, MICROENCAPSULAÇÃO DAS ISOFLAVONAS DO MELAÇO DE SOJA E APLICAÇÃO EM MASSA FRESCA

Santa Maria, RS, Brasil
2018

Vanessa Sabrina Fagundes Batista

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Co-Orientador: Prof. Dr. Cristiano Ragagnin de Menezes

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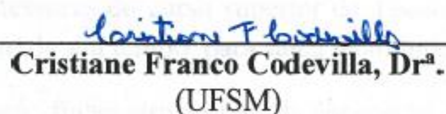

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(UFSM)

Santa Maria, 1º março de 2018

Dedico este trabalho à minha amada,

linda e graciosa filha, Maria Clara,

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"Um cientista em seu laboratório não é somente um técnico, é também uma criança colocada diante de fenômenos naturais que a impressionam como um conto de fadas"

(Marie Curie)

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos
Universidade Federal de Santa Maria

OBTENÇÃO E MICROENCAPSULAÇÃO DE ISOFLAVONAS DO MELAÇO DE SOJA PARA A APLICAÇÃO EM MASSA FRESCA

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Santa Maria, 1° março de 2018.

A soja e seus derivados apresentam potencial no mercado pela presença de compostos bioativos como as isoflavonas. Existem 12 isômeros de isoflavonas sendo considerada biologicamente ativas as isoflavonas nas formas de agliconas. O uso do processo de microencapsulação tem se tornado uma alternativa viável para a incorporação de compostos bioativos em alimentos, por serem encarados como desafio quanto a sua estabilidade em alimentos industrializados. Esse trabalho teve como objetivo extrair as isoflavonas de melaço de soja com diferentes solventes, testar diferentes agentes encapsulantes com diferentes condições de processo e microencapsular o extrato com as melhores características pelo método de *spray drying* e aplicação em massa alimentícia fresca. Foram usados os solventes, etanol e metanol a 80% e álcool de cereais nas concentrações de 50 e 80%, sendo os agentes encapsulantes: 18% maltodextrina DE20 (T1), 18% hi-maize (T2) e uma mistura em proporções iguais de 9% maltodextrina DE20 e 9% de hi-maize (T3) com temperaturas de ar de entrada de 120, 130 e 140 ºC, após escolher o extrato com melhores características foi aplicado em massa fresca na forma de microcápsulas e extrato puro. A separação e quantificação das isoflavonas foram realizadas utilizando cromatográfia líquida de alta eficiência (CLAE), os compostos fenólicos totais foram analisados pelo método de Folin, a atividade antioxidante foi determinada pelos métodos DPPH e ORAC. O álcool de cereais a 50% foi o melhor solvente extrator, apresentando maior rendimento dos compostos bioativos, sendo o amido modificado hi-maize utilizado pelo método de *spray drying* na temperatura de entrada de ar de 130 ºC o melhor agente encapsulante, apresentando menor degradação desses compostos. Nas massas frescas foram avaliadas as características físico-quimicas, estabilidade dos compostos bioativos do produto *in natura* e após o cozimento num período de 21 dias de armazenamento refrigerado 4°C, análises sensorial e microbiológica. As formulações das massas foram, controle (T1), com extrato (T2) e com microcápsulas (T3). As massas cozidas mostraram degradação significativa dos compostos bioativos em relação as massas *in natura* ao longo do armazenamento em todas as formulações, embora os resultado mostram que a massas com as microcápsulas foram mais estáveis que as com o extrato puro, a partir do décimo quarto dia o cozimento das massas degradou totalmente os compostos em todas as formulações. As massas desenvolvidas estão dentro dos Padrões de Identidade e Qualidade de Massas Alimentícias, entretanto a adição de extrato puro na massa alimentícia fresca agiu como um antimicrobiano aumentando a vida de prateleira dessa massa.

Palavras-chave: Compostos bioativos. Antioxidantes. Fenólicos. Estabilidade. *Spray drying*. Massa alimentícia fresca.

ABSTRACT

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ACQUISITION AND MICROENCAPSULATION OF ISOFLAVONES FROM SOYBEAN MOLASSES FOR PASTA APPLICATION

Author: Vanessa Sabrina Fagundes Batista
Advisor: Cláudia Severo da Rosa
Co-Advisor: Cristiano Ragagnin Menezes
Santa Maria, 1° march 2018.

Soy and its derivatives present potential in the market because of the presence of bioactive compounds such as isoflavones. There are 12 isoflavone isomers being considered as biologically active isoflavones in aglycone forms. The use of the microencapsulation process has become a viable alternative for the incorporation of bioactive compounds into foods, to be seen as a challenge as to its stability in processed foods. This study aimed to extract the soy molasses isoflavones with different solvents, to test different encapsulating agents with different air intake temperatures and to microencapsulate the extract with the best characteristics through the spray drying method and application in fresh pasta. The solvents used were 80% ethanol and methanol and grain alcohol in concentrations of 50 and 80%, the encapsulating agents were: 18% maltodextrin DE20 (T1), 18% hi-maize (T2) and a mixture of equal proportions of 9% maltodextrin DE20 and 9% hi-maize (T3) with air intake temperatures of 120, 130 and 140 ºC, after the selection of the extract with the better characteristics, it was applied in fresh pasta in the form of microcapsules and pure extract. The separation and quantification of isoflavones were performed using high performance liquid chromatography (HPLC), the phenolic compounds were analyzed through the Folin method, the antioxidant activity was determined by the DPPH and ORAC methods. The 50% grain alcohol was the best solvent to extract the isoflavones, this extract presented higher yields of isoflavone aglycones, total isoflavones, phenolics and antioxidant activity. The modified hi-maize starch used by the spray drying method at the air intake temperature of 130 ºC was the best encapsulating agent, presenting lower degradation of total and aglycone isoflavones, total phenolics and antioxidant activity. In the fresh pasta it was evaluated the physical and chemical characteristics, the stability of the bioactive compounds of the in natura product and the after cooking in a period of 21 days of refrigerated storage at 4°C sensory and microbiological analyzes as well as the stability of microcapsules throughout storage at room temperature and refrigerated at 4 °C. The formulations of the pastas were, control (T1), with extract (T2) and with microcapsules (T3). The cooked pasta showed a significant degradation of the bioactive compounds in relation to the in natura pasta throughout thestorage in all formulations, although the results show that the masses with the microcapsules were more stable than those with the pure extract, from the fourteenth day the cooking of the masses totally degraded the compounds in all the formulations. The developed pasta are within the Standards of Identity and Quality of Pasta, the psychrotrophic counts increased throughout storage in the control pasta and in those with microcapsules, however the addition of pure extract acted as an antimicrobial, increasing the shelf life of this pasta.

Keywords: Bioactive compounds. Soy Molass. Isoflavones. Microencapsulation. spray drying. Fresh pasta

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1 INTRODUÇÃO

A possibilidade de prevenir e/ou combater doenças por meio da dieta e manter hábitos saudáveis, tem atraído a atenção dos consumidores, tornando um ponto forte a ser abordado por estudos e principalmente de maior interesse para as indústrias de alimentos (REQUE, 2012), em desenvolver os conhecidos alimentos funcionais ou alimentos ricos em um ou mais componentes bioativos (ROBERFROID, 2002), possibilitando elevada qualidade da alimentação humana e ainda agregar valor a alimentos já disponíveis no mercado (XAVIER, 2013).

A soja pertence à família das leguminonas, e é a única fonte proteíca de origem vegetal a ter todos os aminoácidos essenciais. A soja e seu derivados apresentam grande potencial no mercado de alimentos devido à presença de compostos bioativos, como as isoflavonas que tem sido largamente estudadas quanto aos seus efeitos biológicos benéficos à saúde humana (RUIZ-LARREA et al., 1997). Os grãos de soja podem conter até doze tipos de isoflavonas que são encontradas apenas na soja, sendo as principais daidzeína, genisteína e a glicitina, as quais apresentam-se como várias formas de conjugados glicosídeos, dependendo da extensão do processamento ou fermentação dos alimentos, o qual, podem tornar-se um desafio para as indústrias alimentícias, uma vez que, as isoflavonas são instáveis a temperaturas acima de 40ºC, e apresentam baixa hidrossolubilidade e alta taxa de amargor (CARRÃO-PANIZZI et al., 1998; ALDIN, et al., 2006; LARKIN et al., 2008).

O melaço de soja é um subproduto obtido na extração proteica de soja (GAOL, LI & LIU, 2012), Além da necessidade ambiental, reutilizando subprodutos a fim de proporcionar uma diminuição nos custos de produção e utilização total de um alimento (CANTERI et. al., 2008), verifica-se atualmente uma oportunidade de negócios na utilização do melaço de soja, tais como alimentos para animais e ainda na utilização dos compostos com tecnologias simples e eficientes (VENDRUSCOLO & ALBUQUERQUE, 2008; SAID & PIETRO, 2004). Uma vez que, este subproduto apresenta alta concentração de açúcares (57% peso seco), nitrogênio e outros macro e micronutrientes, sendo utilizado principalmente como matéria prima para a fermentação (SIQUEIRA et al., 2008), sendo a produção de etanol combustível o principal destino para o melaço de soja (MACHADO, 1999; SIQUEIRA et al., 2008; LONG & GIBBONS, 2013).

O uso do processo de microencapsulação tem como finalidade proteger os compostos encapsulados contra fatores ambientais e também mascarar características indesejáveis de sabor

e aroma que esses compostos podem conferir nos alimentos, tornado uma alternativa viável para a incorporação de compostos bioativos e aditivos em alimentos quando estes apresentam como um desafio a baixa estabilidade em alimentos industrializados (CHAMPAGNE & FUSTIER, 2007; FAVARO TRINDADE et al. 2008). Portanto, as indústrias alimentícias vêm utilizando o processo de microencapsulação no qual um líquido é atomizado através de uma corrente de ar quente, para obtenção de um pó estantâneo, que pode ser utilizados no desenvolvimento de novos produtos conferindo um aumento do valor nutricional do alimento (GHARSALLAOUI et al., 2007 ; SOBRINHO & FARIAS, 2012).

A massa alimentícia é um alimento com tecnologia simples, de baixo custo, fácil praparo e com alto índice de aceitabilidade (PAUCAR-MENACHO et al., 2008). Com isso, estão definitivamente incoroporadas ao hábito alimentar do brasileiro, sendo consumidas por todas as classes sociais. O consumo per capita de massas frescas, é em média de 5,7 kg/ano no país (ABIMAP, 2016). Sendo muito versátil tanto no ponto de vista nutricional quando no ponto de vista econômico, são apropriadas para serem transformadas em alimentos funcionais e/ou enriquecidos pela incorporação de ingredientes adequados (PRABAHSANKAR et al., 2009).

Sendo assim, a produção de compostos bioativos provenientes da soja, pode ser uma boa alternativa para a incorporação em massa alimentícia fresca, visando a produção de alimentos funcionais e ou enriquecidos com compostos benéficos à saúde. Além disso, o processo de microencapsulação pode auxiliar na emulsão e estabilidade desses combpostos.

2 OBJETIVOS

2.1 Geral

- Extrair isoflavonas do melaço de soja para aplicação em alimentos.
- Caracterizar e microencapsular os extratos de isoflavonas.

2.2 Específicos

- Extrair os compostos bioativos do melaço de soja com diferentes solventes;
- Analisar os compostos bioativos e capacidade antioxidante dos extratos de melaço de soja;
- Quantificar o teor de isoflavonas;
- Microencapsular extrato de isoflavonas pelo método *spray drying*, determinar o melhor agente encapsulante e otimizar as condições do processo;
- Incorporar as microcápsulas em massa alimentícia fresca;
- Avaliar a estabilidade dos compostos bioativos na massa fresca e cozida durante o armazenamento.

3 REVISÃO DA LITERATURA

3.1 GRÃO DE SOJA

O Brasil é o segundo produtor mundial de grão de soja, cerca de 70% do farelo de soja é destinado à exportação e os 30% restantes utilizados em ração animal e uma proporção reduzida como matéria-prima industrial na forma de isolados e concentrados proteicos. O processamento da soja dá origem a diferentes matérias-primas como farinhas de soja, extratos hidrossolúveis e proteínas texturizadas que podem ser utilizados na produção de alimentos que fazem parte da dieta ocidental (WANG et al., 1994; GENOVESE et al., 2002).

Os derivados de soja são amplamente utilizados na indústria como um ingrediente para aumentar o conteúdo de proteínas de muitos alimentos, também possuem propriedades antioxidantes, devido a presença das agliconas, genisteína e daidzeína, que protegem as células dos efeitos prejudiciais de radicais livres (BROUNS, 2002).

Há, entretanto, grande limitação ao uso direto de produtos proteicos derivados de soja na alimentação humana. Essa limitação se deve, principalmente, ao fato de tais produtos apresentarem sabores indesejáveis com relação aos padrões de palatabilidade, comprometendo suas propriedades sensoriais e diminuindo sua aceitabilidade (LIU, 1997). Há dois grupos principais de sabores indesejáveis nos produtos proteicos de soja. Um deles é composto pelos voláteis, responsáveis pelos odores “gramíneo” o outro, pelos compostos não-voláteis, responsáveis pelo gosto amargo e pela adstringência (HSIEH et al., 1981). Uma vez que a principal causa do desenvolvimento de sabor indesejável em produtos proteicos de soja é a ação de lipoxigenases sobre os ácidos linoléico e linolênico (LIU, 1997), a obtenção de cultivares sem as isoenzimas lipoxigenases tem sido a solução mais efetiva para reduzir o sabor indesejável associado a produtos proteicos de soja (NISHIBA et al., 1995; FURUTA et al., 1996; TORRES-PENARANDA et al., 1998).

3.2 MELAÇO DE SOJA

O melaço de soja é obtido do processamento dos grãos de soja na produção de farináceos proteicos, alimentos utilizados especialmente para a alimentação humana (SIQUEIRA et al., 2007). O melaço de soja em geral apresenta aproximadamente 50% de umidade, sendo constituído essencialmente de 60% de carboidratos, 10% de proteínas, 20% de gordura e 10% de cinzas (KINNEY, 2003), como mostra a Tabela 1.

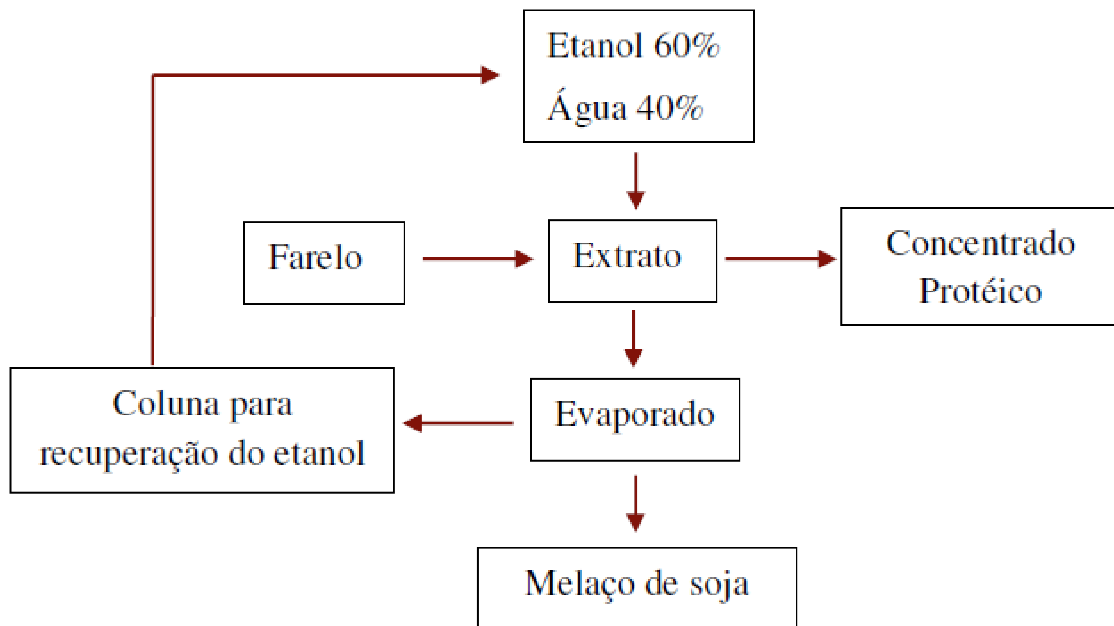
Tabela 1 - Composição do melaço de soja

Componentes	% massa seca
Glicose	0,243
Frutose	0,127
Galactose	0,254
Sacarose	28,4
Rafinose	9,68
Estaquiose	18,6
Proteínas	9,44
Lipídeos	21,2
Fibras	5,7
Cinzas	6,36

Fonte: Siqueira (2007).

A obtenção do melaço de soja consiste na extração hidroalcoólica (60% a 70% de etanol) dos carboidratos a partir do farelo de soja desengordurado para a obtenção de um concentrado rico em carboidratos e com a presença de isoflavonas com valores médios de 60% do total de sólidos solúveis na forma de xarope marrom escuro (JOHNSON et al., 1992; MACHADO, 1999; BARNES et al., 2004). A Figura 1 ilustra o processo de obtenção do melaço de soja.

Figura 1 - Fluxograma obtenção do melaço de soja.



Fonte: adaptado de Siqueira (2007).

A utilização deste subproduto apresenta vantagens econômicas devido à possibilidade de comercialização das isoflavonas pela indústria alimentícia e/ou farmacêutica, uma vez que, parte deste subproduto (melaço de soja) é descartado o que pode causar problemas ambientais (YANG et al., 2009). Além da necessidade ambiental, verifica-se atualmente uma oportunidade de negócios na utilização de resíduos agroindustriais, tais como alimentos para animais e ainda na transformação aos compostos bioativos com tecnologias simples (SAID & PIETRO, 2004; VENDRUSCOLO & ALBUQUERQUE, 2008), sendo a produção de etanol combustível o principal destino para o melaço de soja (MACHADO, 1999; SIQUEIRA et al., 2008; LONG & GIBBONS 2013). Principalmente depois de 2006, com a implantação de usina de álcool na própria planta de uma das maiores beneficiadoras de soja do Paraná. Desde então, o resíduo de melaço de soja gerado, que antes não tinha utilidade e era tratado por queimada (FINEP, 2014), passou a ter utilização na obtenção combustível com valor de mercado.

3.3 ISOFLAVONAS

As isoflavonas fazem parte de uma ampla variedade de compostos fenólicos pertencente ao grupo dos flavonóides de baixa massa molar, denominados metabólitos secundários, são os

maiores componentes fenólicos na soja, sendo encontrados em teores que variam de 0,1 a 5 mg/g (COWARD et al., 1993; GENOVESE E LAJOLO, 2001).

A concentração de isoflavonas nos grãos de soja é geneticamente controlada e influenciada pelas condições ambientais, sendo a temperatura durante o desenvolvimento do grão o fator mais importante (CARRÃO-PANIZZI et al., 1998; TSUKAMOTO et al., 1995). Segundo Carrão-Panizzi et al. (1999), a soja plantada em regiões com temperaturas médias de 20ºC apresentaram concentração média de isoflavonas de 147,8mg/100g (FTAbyara) e 180,1mg/100g (IAS 5) e quando plantadas em regiões com temperatura média de 25ºC apresentaram 73,5mg/100g e 85,5mg/100g, respectivamente.

A presença e a concentração das isoflavonas nos produtos à base de soja também dependem das condições de processamento, principalmente a temperatura de tratamento que o alimento sofre (COWARD et al., 1998; WANG et al., 1996). Produtos não-fermentados possuem concentrações de isoflavonas duas a três vezes maiores que produtos fermentados (WANG et al., 1996). Entretanto, a distribuição dos constituintes difere nestes dois grupos: produtos fermentados apresentam predominantemente agliconas enquanto os produtos não-fermentados apresentam maiores concentrações de β-glicosídios (COWARD et al., 1993). Segundo Song et al. (1998), o teor de isoflavonas na maioria dos alimentos à base de soja varia de 100 a 300mg/100g.

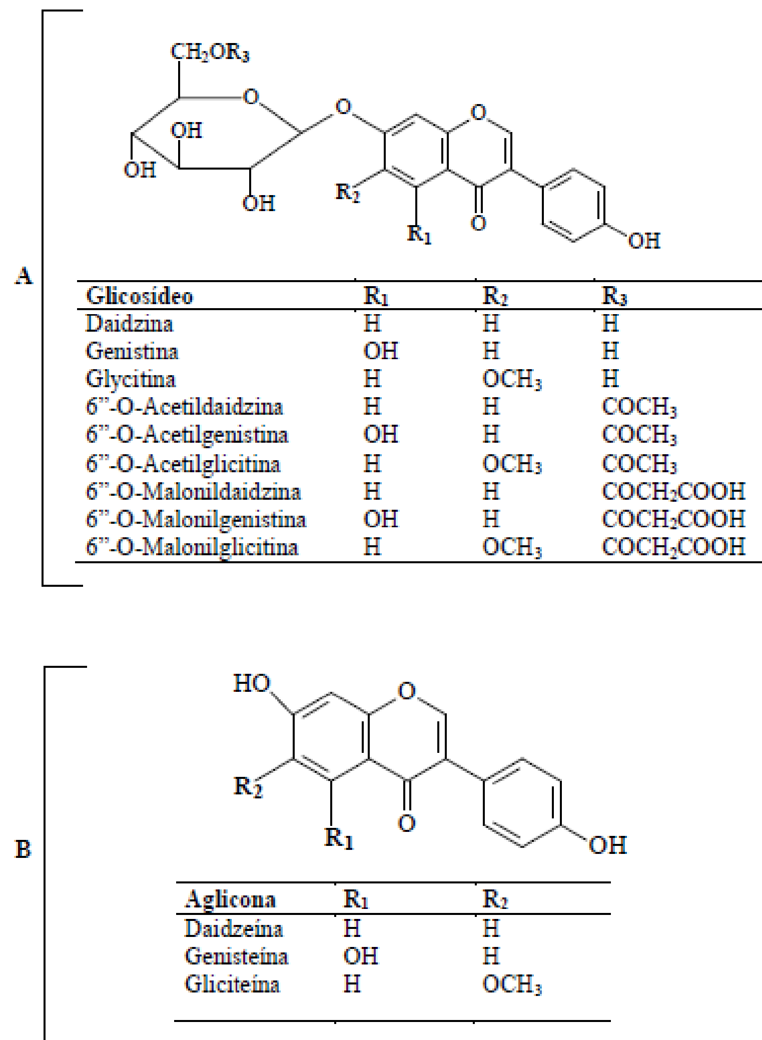
As isoflavonas isoladas apresentam doze componentes, sendo malonil derivados e β-glicosídeos (Figura 2A), que ocorrem naturalmente nos grãos da soja e na farinha de soja e os acetil derivados e as agliconas (daidzeína, genisteína e gliciteína) (Figura 2B), que são formadas durante o processamento industrial da soja ou no metabolismo da soja no organismo (CHUNG et al., 2014; KIM et al., 2012).

A ingestão diária de isoflavonas nas formas agliconas (daidzeína, genisteína e gliciteína) apresentam maior atividade biológica em relação às formas glicosídicas (PARK et al., 2001; SONG et al., 1998) e garantem efeitos benéficos incluindo atividade antioxidante, anti-inflamatória, antimicrobiana e anti-carcinogênica (BEER et al., 2005; DELMAS et al., 2005; IKEDA et al., 2006; OSOSKI & KENNELLY, 2003). Estudos clínicos e epidemiológicos também mostraram uma baixa incidência de osteosporose e certos tipos de câncer relacionada ao alto consumo de soja (LEE et al., 2003; SARKAR & LI, 2003).

O órgão fiscalizador *Food and Drug Administration* (FDA, 1999) concedeu o certificado *Generally Recognized as Safe* (GRAS) às isoflavonas obtidas de fontes naturais (JACKSON et al., 2002). Porém para alimentação infantil algumas preocupações foram relatadas em relação a seus efeitos negativos no desenvolvimento e reprodução sexual,

desenvolvimento neurocomportamental, imunidade e tireoide (CEDERROTH; ZIMMERMANN; NEF, 2012).

Figura 2 - Estruturas das isoflavonas da soja: (A) glicosídeos e (B) agliconas.



Glicosídeo	R_1	R_2	R_3
Daidzina	H	H	H
Genistina	OH	H	H
Glycitina	H	OCH_3	H
6”-O-Acetildaidzina	H	H	$COCH_3$
6”-O-Acetilgenistina	OH	H	$COCH_3$
6”-O-Acetilglicitina	H	OCH_3	$COCH_3$
6”-O-Malonildaidzina	H	H	$COCH_2COOH$
6”-O-Malonilgenistina	OH	H	$COCH_2COOH$
6”-O-Malonilglicitina	H	OCH_3	$COCH_2COOH$

Aglicona	R_1	R_2
Daidzeína	H	H
Genisteína	OH	H
Gliciteína	H	OCH_3

Fonte: Jackson et al. (2002) com modificações.

Quando esses compostos são ingeridos, vão ser metabolizados por bactérias no intestino grosso e formam o equol, que se assemelha à estrutura química do estradiol (estrógeno humano), o que pode ser observado na Figura 3.

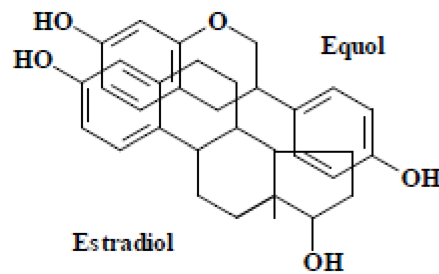


Figura 3 - Estruturas moleculares do equol e estradiol
Fonte: Murkies et al. (1988), com modificações.

Diferentemente das demais formas das isoflavonas, as agliconas podem ter seu conteúdo aumentado, quando o tratamento hidrotérmico dos grãos de soja for realizado com temperatura ótima para ativar a enzima beta-glicosidase (CARRÃO-PANIZZI et al., 2004). A presença dessa forma de isoflavonas biodisponível é importante no processamento de alimentos funcionais de soja, os quais são dirigidos para consumidores preocupados com a saúde.

3.3.1 Benefícios à saúde

As isoflavonas tem uma ampla gama de mecanismos de ação, uma vez que, suas propriedades biológicas se estendem ainda mais além de sua capacidade de se juntar aos receptores de estrogênio, incluindo propriedade antioxidante, pois impossibilitam a produção de oxigênio reativo na formação de radicais livres, tornando mais lenta a taxa de oxidação do colesterol LDL (SILVA et al., 2009), na regulação da atividade de algumas enzimas, prevenção do câncer, doenças cardiovasculares, patologia nas alterações do sistema endócrino (ADLERCREUTZ et al. 1998). Decorrente disso, seu efeito clínico tem variação individual mesmo quando controlada a quantidade de isoflavonas administrada, sendo difícil estabelecer a dose ideal, no entanto recomenda-se uma dose diária de 20 a 100mg/dia de isoflavonas. (BELLEI, 2010).

Considerando que as isoflavonas possuem efeitos estrogênicos como antiestrogênicos, de acordo com alguns estudos ainda é difícil de determinar se as isoflavonas protegem ou estimulam o câncer de mama. Alguns autores sugerem que estes compostos agonistas e antagonistas podem modular o risco de câncer de mama, quando consumido antes da puberdade. Como a puberdade poderia ser um fator de risco para alguns tipos de câncer associados a hormônios, como o câncer de mama, incluindo o câncer de próstata e testicular, houve um grande interesse em estudar os efeitos fitoestrógenos das isoflavonas.

Rice et al. (2006), ao realizar um estudo com meninas saudáveis de 9 anos de idade, observou um atraso no desenvolvimento de mama, associado a uma ingestão diária de 2mg de isoflavona. Meninas com 2 anos de idade ao ingerirem 1,2 mg/dia de isoflavonas, apresentaram um atraso de desenvolvimento de mamas em aproximadamente 7 meses, apresentando uma velocidade alta na faixa de crescimento em altura. Esse mesmo estudo realizado em meninos com idade semelhante, recebendo ingestão diária de 1,4 mg de isoflavonas não afetou o momento da puberdade.

Silva et al. (2009) associaram a ingestão de soja com a diminuição de episódios de câncer e, ainda, sugeriram que o consumo em doses altas está relacionado com diminuições de concentrações do colesterol sérico. Várias pesquisas com animais têm demonstrado eficácia, com consequente redução de aterosclerose em animais tratados com uma dieta à base de proteínas de soja quando comparada a uma dieta baseada em proteínas animais.

Estudos realizados por Tang et al. (2006), com mulheres de 45 a 60 anos, verificaram a atuação de isoflavonas sobre o tecido ósseo, numa ingestão diária de 84 a 126mg de isoflavonas, num espaço de tempo de seis meses, onde percebeu-se um elevado percentual de mudanças na densidade mineral óssea (DMO) na coluna lombar e no colo femoral.

Estudos realizado por Silva et al. (2013), comprovam um resultado positivo e relevante sobre os sintomas climatéricos em mulheres, conhecidos como os fogachos, ondas de calor. Verificaram que podem ser amenizados e/ou diminuídos esses sintomas com o uso de isoflavonas, que interfere positivamente em mulheres na menopausa, além de refletir positivamente na qualidade de vida destas pessoas.

3.4 MICROENCAPSULAÇÃO

A microencapsulação na indústria de alimentos tem como finalidade proteger os ingredientes encapsulados contra a oxidação química, de fatores ambientais como temperatura, luz, oxigênio. Também pode ser utilizado para a proteção de compostos que sejam desejáveis nos alimentos como vitaminas, polipeptídios e compostos bioativos nos quais se enquadram vitaminas, minerais, probióticos, fitoesteróis, luteína, ácidos graxos, licopeno e antioxidantes, durante todo processamento, estocagem e transporte (CHAMPAGNE & FUSTIER, 2007; DE VOS et al., 2010; FANG & BHANDARI, 2010; SOBRINHO & FARIAS, 2012; MENEZES et al., 2013; DALLAGNOL, 2013).

A microencapsulação foi desenvolvida há aproximadamente 60 anos (FANG & BHANDARI, 2010) e pode ser definida como a tecnologia utilizada para recobrir pequenas

partículas de material sólido, líquido ou gasoso, onde as mesmas são aprisionadas em filmes finos, formando pequenas cápsulas de um agente de microencapsulação de grau alimentar, as quais podem liberar o seu conteúdo de forma controlada ou sob condições específicas (GHARSALLAOUI et al., 2007; NAZZARO et al., 2012).

Shahidi e Han (1993) propuseram seis razões para a aplicação de microencapsulação na indústria de alimentos:

- Reduzir a reatividade do núcleo com fatores ambientais;
- Diminuir a taxa de transferência do material do núcleo para o ambiente exterior;
- Promover a manipulação de forma mais fácil;
- Controlar a liberação do material do núcleo;
- Mascarar o sabor do núcleo;
- Meio de diluição do material do núcleo quando o mesmo deve ser utilizado em pequenas quantidades.

Segundo Gharsallaoui et al. (2007) os diferentes tipos de microesferas e microcápsulas são produzidos através de uma vasta gama de materiais de parede entre eles açucares, gomas, proteínas, polissacarídeos naturais e modificados, lipídios e polímeros sintéticos (FANG & BHANDARI, 2010). A microencapsulação conta com um grande número de processos, tais como: *spray drying, spray cooling*, refrigeração, coacervação, extrusão, liofilização, extrusão centrífuga, co-cristalização, recobrimento em leito fluidizado, lipossomas e complexação por inclusão, estes métodos têm sido empregados na elaboração das microcápsulas (FANG & BHANDARI, 2010; SOBRINHO & FARIAS, 2012; NAZZARO et al., 2012).

Os materiais a serem encapsulados podem ser puros ou com misturas e podem ser chamados de núcleo, fase interna, material revestido, preenchimento da fase interna ou carga útil. Os materiais de embalagem são chamados de material de revestimento, escudo, material de parede ou membrana, transportador, concha ou cápsula (GHARSALLAOUI et al., 2007; FANG E BHANDARI, 2010).

Muitas técnicas de microencapsulação foram desenvolvidas ao passar dos anos, no entanto, para microencapsulação de ingredientes alimentares a técnica de *spray drying* é a mais comumente utilizada devido ao seu baixo custo e equipamentos prontamente disponíveis (GHARSALLAOUI et al., 2007; NAZZARO et al., 2012; SIMEONI et al., 2014). A microencapsulação por *spray drying* é um dos processos mais antigos de encapsulamento, sendo este utilizado desde 1930 para preparação dos primeiros aromas encapsulados usando goma arábica como material de parede (GHARSALLAOUI et al., 2007).

O método de *spray drying* (figura 4) é considerado uma operação unitária no qual um produto líquido é atomizado através de uma corrente de gás (ar ou ozônio) quente para obtenção de um pó instantaneamente. O líquido inicial do pulverizador pode ser uma emulsão ou suspensão. A secagem por pulverização pode produzir um pó muito fino (10 a 50 μm) ou grandes partículas (2 a 3 mm), este fator vai depender das condições de alimentação do material de partida e de funcionamento do equipamento em questão (GHARSALLAOUI et al., 2007; SIMEONE et al., 2014).

Existem etapas básicas a serem seguidas para o processo de microencapsulação por *spray drying,* primeiramente é necessário que seja realizada a preparação da dispersão ou emulsão a ser processada, em um segundo momento faz-se a homogeneização da dispersão e como procedimento final tem-se a atomização da massa dentro da câmara de secagem (CALEFFI, 2014).

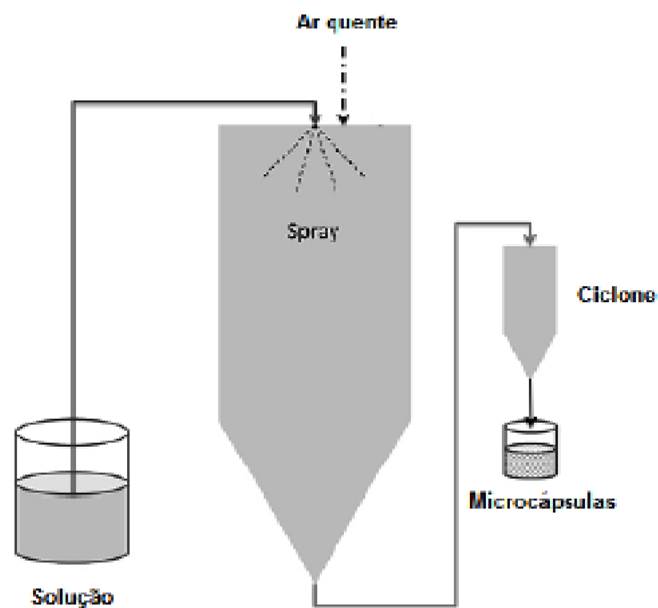


Figura 4 – Esquema da microencapsulação por *spray drying*
Fonte: Estevinho et al., (2013).

3.4.1 Agentes encapsulantes

Para iniciar o processo de encapsulação o primeiro passo é a escolha do agente encapsulante adequado. Esta escolha depende de uma série de fatores como: apresentar propriedades emulsificantes, não apresentar reatividade com o material a ser encapsulado, ser capaz de formar filmes, ser biodegradável, apresentar resistência ao trato gastrointestinal, baixa viscosidade e ser de baixo custo, associado à escolha do processo de microencapsulação e o mecanismo de liberação ideal (PEGG & SHAHIDDI, 2007; SOBRINHO & FARIAS, 2012; DALLAGNOL, 2013; SIMEONE et al., 2014).

Agentes encapsulantes são substâncias que formam uma película em torno do composto desejado, proporcionando uma entrega melhorada de ingredientes funcionais (SOBRINHO & FARIAS, 2010). Para atuar como emulsificante, um composto deve conter grupamentos hidrofílicos e hidrofóbicos. Quanto melhor for a capacidade emulsificante do composto melhor será a retenção de compostos (ROCHA, 2009).

Segundo Shahidi e Han (1993) os materiais encapsulantes mais utilizados compreendem:

- Carboidratos: amidos, dextrinas, xarope de milho, sacarose;
- Celulose: carboximentil celulose, etil, metil, acetil e nitro-celulose;
- Gomas: goma arábica, guar, alginato de sódio, carragena;
- Lipídios: cera, parafina, triestearina, ácido, mono e diglicerídeos e gorduras hidrogenadas;
- Proteínas: glúten, caseína, isolado proteico de soro de leite, gelatina e albumina, algumas fontes alternativas como quitosana.

A maltodextrina é caracterizada por sua dextrose equivalente (DE) e por seu grau de polimerização (DP). Assim as propriedades das maltodextrinas estão associadas ao DE e ao DP, que variam de acordo com o grau de hidrólise durante o processamento do amido (BICUDO, 2014).

A maltodextrina é definida como todo o amido hidrolisado com valores de dextrose equivalente entre 3 e 20. O valor de DE reflete na sua estabilidade e funcionalidade (CALEFFI, 2014). Apresenta-se como um pó branco ou solução concentrada, formada pela hidrólise parcial do amido de milho com ácidos e/ou enzimas. Não possui propriedades emulsificantes (hidrofílica e lipolítica), por este motivo deve ser utilizada com amidos modificados para a estabilização de emulsões (CALEFFI, 2014; BICUDO, 2014).

Amidos modificados são considerados de baixo custo e apresentam excelente propriedade emulsificante. A utilização de amidos modificados para encapsulação tem sido relacionada em sua maioria para a encapsulação de aromas (PARAMERA et al., 2011), não sendo encontrados muitos estudos quanto a sua utilização como material de parede para compostos bioativos com aplicação em alimentos.

Segundo o material publicado pela Federação das Indústrias do Estado de São Paulo (FIESP) em parceria com o Instituto de Tecnologia de Alimentos (ITAL), Brasil Food Trends 2020, os amidos modificados são importantes agentes de textura e de resistência à variação de temperatura (FIESP; ITAL, 2010). Porte et al. (2011) afirmam que o amido modificado possui propriedade de redução da tensão superficial de uma solução podendo ser utilizado como emulsificante.

Sobrinho e Farias (2012) relatam que dificilmente um agente encapsulante irá apresentar isoladamente todas as propriedades para a formação de uma cápsula resistente e adequada para o que se deseja, por este motivo é comum o emprego de misturas de materiais encapsulantes.

3.5 MASSA FRESCA

A massa alimentícia é um alimento básico em muitos países, em 100 indivíduos, 97% assume ser consumidor frequente (LEMES et al., 2012). No brasil, foi incorporado na culinária há muitas décadas, tornando consumo de massas alimentícias bastante significativo, com um consumo per capita de massas frescas em média de 5,7 kg/ano no país (ABIMAP, 2016). Sendo uma fonte moderada de proteínas, vitaminas e hidratos de carbono complexos (BOROSKI et al., 2011), é de fácil e rápida preparação, podendo servir como prato principal ou complemento (MATSUO et al., 1992).

Sendo um produto de baixo custo e fácil aceitação, devido à sua rápida confecção, são excelentes para a adição de ingredientes funcionais. Sendo um produto não fermentado, possui várias formas, com ou sem recheio (PAUCAR-MENACHO et al., 2008). As massas alimentícias são definidas pela legislação brasileira como produtos obtidos pelo amassamento mecânico de farinha de trigo (*Triticum aestivum L.*) e/ou de outras espécies do gênero Triticum e/ou derivados de trigo durum (*Triticum durum L.*) e/ou derivados de cerais leguminosas raízes tubérculos com ou sem adição de ingredientes, com ou sem tempero ou suplemento (ANVISA, 2006).

As massas alimentícias são uma opção nutricional e, apesar de sua carência em alguns nutrientes indispensáveis à alimentação humana, as massas alimentícias ajudam a compor a

base da pirâmide alimentar por serem ricas em carboidratos energéticos (HIBIG et al., 2007). Por isso, essas deficiências podem ser compensadas com outros ingredientes tornando-as apropriadas para serem transformadas em alimentos funcionais e/ou enriquecidos através da incorporação de ingredientes adequados (PRABHASANKAR et al., 2009).

4 MANUSCRITOS

4.1 Manuscrito 1

EXTRACTION, CHARACTERIZATION, AND MICROENCAPSULATION OF PHENOLICS FROM SOYBEAN MOLASSES[1]

[1]Artigo formatado de acordo com as normas da revista *Food Research International*.

Extraction, characterization and microencapsulation of phenolics from soybean molasses

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Abstract

This study aimed to extract the phenolics from soybean molasses with different solvents, microencapsulate the extract with better characteristics by testing different encapsulating agents in order to increase the stability. Solvents, ethanol and methanol at 80% and grain alcohol in concentrations of 50 and 80% were used, the encapsulating agents were: 18% Maltodextrin DE20 (T1), 18% Hi-maize (T2) and a mixture in equal proportions of 9% Maltodextrin DE20 and 9% of Hi-maize (T3). The 50% grain alcohol was the best phenolic extractive solvent. The modified Hi-maize starch using the air inlet temperature in the spray drying at 130 ºC was the best encapsulating agent, presenting higher phenolic retention. Residues such as soybean molasses have compounds of great interest, such as isoflavones, therefore it is increasingly necessary to the research investment in order to increase the yield of the extraction of these compounds together with encapsulating agents suitable to retain the compounds of interest.

Keywords: *Spray-drying*. Antioxidant. Phenolics. Stability. Isoflavones. Grain alcohol**.**

32 **1 Introduction**

33

34 Soy is one of the most important products in the Brazilian economy, holding a prominent
35 place in the export agenda of the country. Production is estimated at 116.996 million tons in the
36 2017/2018 harvest, placing Brazil as the second biggest grain production (CONAB, 2018),
37 which on the other hand produces a significant amount of waste, whose disposal in the
38 environment, in the long term can generate irreversible damages.
39 Due to the environmental impacts of the by-products generation, there is currently a
40 need to reuse these by-products in order to provide a reduction in production costs and total
41 utilization of food (Canteri, Loss & Barana, 2008). Molasses is produced concurrently with the
42 soybean protein concentrate, a dark brown syrup rich in carbohydrates and isoflavones
43 (Johnson, Myers & Burden, 1992). In addition, it is used as raw material for fermentation
44 (Siqueira et al., 2008), with fuel ethanol production being the main destination (Machado, 1999;
45 Siqueira et al., 2008; Long & Gibbons, 2013). Since it is an agroindustrial waste with a high
46 volume of generation, it is also a material of low commercial cost that imposes problems of
47 environmental discarding, becoming a by-product with great potential of reutilization in the
48 market, aimed at obtaining functional foods, due to the presence of bioactive compounds.
49 The soybean grain has high isoflavone contents, varying from 12 to 461 mg/100 g and
50 being the main phenolics found in this legume. The content of isoflavones can be influenced by
51 environmental factors and planting region. Among the factors, the temperature is the most
52 important one (Carrão-Panizzi, Berhow, Mandarino & Oliveira, 2009). Soy isoflavones have
53 different beneficial effects on human health, such as anti-carcinogenic activity, antioxidant
54 activity, anti-inflammatory activity and a decrease in serum cholesterol (Liang, Wang, Li, Hao
55 & Wang, 2010; Barbosa, Lajolo & Genovese, 2011).
56 The use of bioactive compounds alone in food is a challenge for the food industry due
57 to its instability. Microencapsulation process is intended to protect the encapsulated compounds
58 against environmental factors and the components of the food itself, as well as to mask
59 undesirable flavor and aroma characteristics that these compounds can confer on food. The
60 incorporation of bioactive compounds and encapsulated food additives has become a viable
61 alternative once it allows the delay of alterations that result in loss of aroma, changes in color
62 and loss of the nutritional value of these compounds and of the food itself.
63 The microencapsulation process makes it possible to reduce the rate of migration of the
64 material from the core to the external environment. (Champagne & Fustier, 2007; Favaro
65 Trindade, Pinho & Rocha, 2008). The method of microencapsulation by spray drying consists

of a suspension, which is atomized through a stream of hot air, to obtain an instant powder which can be used in the development of products conferring a nutritional increase of the food (Gharsallaoui, Roudant, Chambin, Voilley & Saurel, 2007; Sobrinho & Farias, 2012). The type of encapsulating agent is one of the major factors influencing the stability of the encapsulated compound (Suave et al., 2006), mainly on the characteristics as intended application, selected microencapsulation method and, in addition, mainly on the physical and chemical characteristics of the bioactive compound (Jain, 2004).

Considering the importance of the isoflavones derived from soybean, and the microencapsulation process that allows to confer stability to the extracted compound maintaining its functional properties during the application in foods, this study had the following objectives: to test different solvents to extract the phenolics from soybean molasses; to quantify the isoflavone content; to test different encapsulating agents and to microencapsulate the extract with the best characteristics by the spray drying method for food application.

2 Materials and methods

2.1 Extraction of phenolic compounds

Soy molasses was supplied by the Selecta industry (Goiânia, GO). Three solvent systems were tested: 80% methanol 1:10 (sample/solvent, v/v) (Fukutake, Takahashi, Ishida, Kawamura, Sugimura & Wakabayashi 1996), 80% ethanol 1:10 (sample/solvent, v/v) (Pereira, Seixas & Neto, 2002) and 80% and 50% grain alcohol 1:10 (sample/solvent, v/v). The extraction was performed according to Carrão-Panizzi, Simão and Kikuch (2003), with modification. The extracts were placed under constant agitation for 2 hours at room temperature (shaker at 250 rpm - Orbital Tecnal Mod. TE140), followed by centrifugation (Servilab MTD III Plus centrifuge) at 1083 g for 20 min to obtain two fractions: the supernatant and the precipitate. The supernatant was collected and the precipitate was subjected to exhaustive extraction for a further 2 hours, totalling a 4 hour extraction. After exhaustive extraction, the extracts were submitted to the drying process by rotaevaporation at 40ºC for solvent evaporation and extract concentration, to identify the isoflavones by high performance liquid chromatography (HPLC) and subsequently subjected to the microencapsulation process. For the other analyses, the extracts were stored under refrigeration.

100 *2.2 Determination of total phenolic compounds*

101

102 The determination of the phenolic content was set by the Folin-Ciocalteu method
103 described by Singleton, Orthofer and Lamuela-Raventos (1999), with modifications by Roesler
104 (2007). For the colorimetric reaction, 0.4 mL of the extracts previously diluted in the extraction
105 solvent were transferred to the test tubes, after which 2.0 mL of 0.2 N aqueous *Folin-Ciocalteau*
106 solution (diluted 1:10) was added. After shaking, the tubes were left to stand in the absence of
107 light for three minutes. Then, 1.6 mL of sodium carbonate (Na_2CO_3) at 7% (m/v) were added
108 and the same incubated for 5 min in water bath at 50 °C. The reading was performed in a
109 spectrophotometer at 760 nm. Total phenolic compound contents were expressed as milligrams
110 of gallic acid/ 100 g of soybean molasses extract (mg GAE/100 g).

111

112 *2.3 Free radical sequestration (DPPH)*

113

114 The antioxidant capacity was determined by the reduction of the stable radical 2,2-
115 diphenyl-1-picryl-hydrazyl (DPPH) according to the methodology proposed by Brand-
116 Willams, Cuvelier and Berset (1995). Aliquots of the extract were diluted according to each
117 solvent used for each extraction type, followed by the addition of 2.5 mL of a 0.1 mM DPPH
118 methanolic solution with 0.5 mL solutions containing increasing concentrations of the extracts
119 and incubated for 30 min in the absence of light. A "control" solution was performed with 2.5
120 mL of the methanolic solution of DPPH in 0.5 mL methanol replacing the extract and the
121 "blank" using methanol. The readings were performed in a spectrophotometer (BEL Photonics
122 - 1105) at the wavelength of 517 nm. The ability to sequester free radical was calculated
123 according to equation 1 and expressed as percentage of inhibition of oxidation of the radical.

124 $$\% \text{ Inhibition} = ((Abs._{DPPH} - Abs._{Am/Trolox}) / Abs._{DPPH}) * 100 \qquad \text{Equation (1)}$$

125 Where $Abs._{DPPH}$ is the absorbance of the DPPH and $Abs._{Am/Trolox}$ is the absorbance of
126 the sample or standard in solution. The $Abs._{Am/Trolox}$ was calculated based on the difference in
127 absorbance of the test sample solution with its blank.

128 The IC_{50} value was determined by the equation of the line plotted through the results
129 containing the concentration values (mg/mL) used in the X axis and the percentages of
130 protection found in the Y axis. A standard Trolox curve in µmol versus % of inhibition was also
131 constructed, where the result was expressed in µmol equivalents of Trolox/g sample (µmol
132 TEAC/g).

133

134 *2.4 The oxygen radical absorption capacity (ORAC)*

135

136 The antioxidant capacity was determined by the ORAC method (Oxygen Radical
137 Absorption Capacity) proposed by OU, Hmpsch-Woodill and Prior (2001). This method
138 verifies the efficiency of an antioxidant under a peroxyl radical induced by AAPH at 37 °C. In
139 a 96-well microplate, 25 µL of the diluted extract (200 mg mL^{-1}) was added in phosphate buffer,
140 pH 7.4 (75 mmol L^{-1}), followed by incubation in a microplate reader (HIDEX, Turku , Finland)
141 for 10 min at 37 °C with 150 µl fluorescein working solution (81 nmol L^{-1}). After incubation,
142 25 µl of AAPH (152 mmol L^{-1}) were added to form peroxyl radicals. Fluorescence was
143 monitored every minute (excitation and emission wavelengths of 485 and 528 nm, respectively)
144 for 121 min at 37 °C. The area under curve (AUC) was calculated by the fluorescence decay
145 over time in the presence of an antioxidant agent and the value corresponding to white (without
146 antioxidants) was subtracted, obtaining the net AUC. The results were compared with a
147 standard Trolox curve (0-96 µmol L^{-1}) and expressed as µmol of Trolox equivalent to 1 g of
148 sample. The AUC was calculated as follows:
149 Where,

150 $$AUC = 1 + {f1}/{f0} + {f2}/{f0} + {f3}/{f0} + \dots {fn}/{f0}$$

151 f **1**...fn: fluorescence determined every minute.
152 f0: fluorescence at time zero.

153

154 *2.5 High performance liquid chromatography (CLAE)*

155

156 The isoflavones were analysed using a high performance liquid chromatography (LC-
157 20A Prominence, Shimadzu, Japan) equipped with a quaternary pump (LC-20AD), manual
158 injector (CTA-20A) and diode arrangement detector (SPD-M20A). The data were processed in
159 the LC solutions program (Version 3, Shimadzu, Columbia, U.S.A). Separation of the
160 compounds was performed on reverse phase column (ODS C18 Microsorb-Mv, 25 mm x 4.6
161 mm - 5 µm) in gradient elution with flow rate of 0.8 mL / min. Acidified water (0.1% acetic
162 acid) (A) and acidified methanol (0.1% acetic acid) (B) were the solvents used as the mobile
163 phase. The gradient was 0 min, 30% B, in 7 min 40% B, in 15 min 50%, in 25 min 50% B,
164 according to the methodology proposed by Rostagno, Malpa and Barroso (2005).
165 Prior to the injection, samples were treated for the removal of interferences in polymer
166 resin cartridges (500 mg, 6cc, Dionex™ SolEx™ HRPHS Polymer-Based SPE Cartridge,

167 Thermo Fischer Scientific) for solid phase extraction as described by Kledjus, Vitamvásová
168 and Kubán (1999), with modifications. Prior to injection, all samples were concentrated in
169 rotaevaporator (Buchi R3) and diluted in the initial mobile phase as required. Samples were
170 injected in duplicate using a volume of 50 μl. Samples were filtered on a PTFE syringe filter
171 (0.45 μm, Millipore) prior to injection. The identification of isoflavones was obtained by
172 comparing retention times and spectra in the ultraviolet (UV) range to the visible of the
173 separated compounds with the authentic standards available. Quantification was performed by
174 integrating peak areas at 254 nm. The limits of quantification (LoQ) and detection (LoD) of the
175 method were: LoD = 0.78 and LoQ 2.36 for daidzein and LoD = 0.29 and LoQ = 0.89 for
176 genistein determined by curve data analysis, which were used to quantify the different aglycone
177 isoflavones present in soybean molasses. All compounds with UV-visible spectra similar to that
178 expected for isoflavones and the standards used in this work were considered as compounds
179 derived from this class and totalized for determination of the total isoflavone concentration in
180 the sample. All compounds with UV-visible spectra similar to that expected for isoflavones and
181 the standards used in this work were considered as compounds derived from this class and
182 totalised for determination of the total isoflavone concentration in the sample.
183
184 *2.6 Microencapsulation of the isoflavone-containing extract*
185
186 *2.6.1 Preparation of microcapsules*
187
188 Microencapsulation was performed on a laboratory scale using mini Spray drying
189 (Buchi, B-290) with 1.5 mm feed nozzle with a flow rate of 0.45 L/h. A percentage of 50%
190 grain alcohol 1:10 (sample/solvent, v/v) was used as the standard solvent and the isoflavones
191 extracts were mixed with maltodextrin MOR-REX®192, DE20 and Hi-maize® 260
192 (22000b00) (modified starch) at different concentrations. Treatment 1 corresponds to 18%
193 maltodextrin (T1), treatment 2 to 18% modified starch (T2), treatment 3 to a mixture of equal
194 portions of maltodextrin and 9% modified starch each (T3). All treatments were tested at
195 different drying temperatures (inlet air) of 120, 130 and 140 °C.
196
197 *2.6.2 Microencapsulation efficiency*
198

199 The microcapsules were dissolved by the method proposed by Robert et al. (2010), with
200 modifications. A total of 0.6 g of capsules were weighed and 3 mL of acetonitrile and 3 mL of
201 a methanol:acetic acid:water (50:8:42 v/v/v) solution were added. The mixture was agitated for
202 1 min in vortex, and then placed in an ultrasonic bath for 60 min, centrifuged at 500 rpm/ 15
203 min.
204 The encapsulation efficiency was evaluated by the phenolic compounds content of the
205 microcapsules. The determination of surface phenolic compounds followed the methods
206 described by Robert et al., (2010), with modifications. A number of 0.4 g of microcapsules were
207 weighed for each formulation and 2 mL of an ethanol:methanol (1:1 v/v) mixture was added
208 followed by the procedures established in item 2.6.2. Surface phenolic compounds (SFC) and
209 encapsulation efficiency (EE) were calculated according to equations 1 and 2, respectively.

210 $$SFC\ (\%) = \frac{Surface\ phenolic\ compounds}{Theoretical\ content\ of\ phenolic\ compounds}\ x\ 100 \quad (1)$$

211 $$EE\ (\%) = 100 - SFC(\%) \quad (2)$$

212

213 *2.7 Statistical analysis*

214

215 Data were submitted to analysis of variance (ANOVA), followed by the Tukey test for
216 comparison of means. The results were considered significant when $p < 0.05$. Statistical analyses
217 were performed in the STATISTICA version 7.0 (StatSoft Inc, Tulsa - OK, USA).

218

219 **3 Results and discussion**

220

221 *3.1. Total phenolics, antioxidant activity and isoflavones quantification in soybean molasses*
222 *extract*

223

224 Table 1 shows the effect of different solvents on the extraction of the phenolic
225 compounds present in soybean molasses.
226 Table 1
227 The results show that 50% grain alcohol extraction (v/v) (244.89 ± 11.20 mg GAE/100
228 g) was better for the extraction of phenolic compounds from soybean molasses, differing
229 statistically from the other solvents. These values were higher than the ones found by
230 Mantovani, (2013), in which phenolics ranged from 26.8 (± 3.6) to 45.7 (± 1.9) mg GAE/100 g
231 and 28.3 (± 3.1) to 59.8 (± 2.3) mg GAE/100 g of soy molasses, using as solvent 80% methanol

232 and 90% ethanol, respectively. The same author found values for total phenolics that ranged
233 from 27.6 (± 1.9) to 59.0 (± 2.2) mg/100 g of soy molasses when using only water as solvent.
234 According to Naczk and Shahidi (2003), there is no solvent extraction system
235 satisfactory for the isolation of all bioactive compounds, because different factors, such as
236 amount of bioactive compounds in foods, variation of polarities between solvents and
237 possibility of interaction with carbohydrates, proteins and other food components may interfere.
238 Studies performed by Menezes, Barin, Cichoski, Zepka, Jacob-Lopes and Terra (2013)
239 showed that pure water is not efficient to extract phenolic substances and, in addition, that
240 mixtures of alcoholic solvents with water are more efficient. The mixture of water with grain
241 alcohol provided an increase in the polarity of the extracting solvent, thus higher phenolic
242 compound contents can be extracted (Sultana, Anwar & Przybylski, 2007; Wijekoon, Bhat &
243 Karim, 2011; Hussain & Chakraborty, 2012); which are results similar to those found in this
244 study.
245 The forms of isoflavones vary from compound to compound (Whang & Murpy, 1994),
246 Ruiz-Larrea et al. (1997) observed that the antioxidant capacity of isoflavones is associated
247 with the number of hydroxyls, which follows the decreasing order of genistein, daidzein,
248 genistein and daidzin with the lowest antioxidant capacity. The results obtained in this study
249 show that the antioxidant capacity of soybean molasses extracts, determined by the IC_{50} (Table
250 1) with 50% grain alcohol as the solvent, showed genistein values (4.25 ± 0.53 mg/100 g of
251 soybean molasses) and daidzein (15.38 ± 1.50 mg/100 g) (Table 2) were higher when
252 compared to the isoflavones aglycones found with the other solvents. According to Arbos,
253 Feitas, Stertz and Dornas (2010), the higher the antioxidant activity, the lower the IC_{50} values,
254 so that values above 25 mg/g are considered to have low antioxidant potential. Thus, it is
255 observed that extracts obtained with grain alcohol showed higher antioxidant activity,
256 differing statistically from the extracts obtained by the ethanol and methanol solvents. The
257 same occurs for the values obtained by the ORAC method in the different solvents.
258 Table 2
259 The levels of daidzein and genistein (15.38 ± 1.50 and 4.25 ± 0.53, respectively) were
260 higher with 50% grain alcohol extraction (Table 2). This study presents high results when
261 compared to those found by Mantovani, (2013), who used ultrafiltration of isoflavone
262 aglycones from soybean molasses, resulting in values of daidzein 1.70 (± 1.3) mg/100 g and
263 genistein 0.014 (± 1.2) mg/100 g of soybean molasses, with the use of 80% methanol as solvent
264 extractor. Figure 1 shows the chromatograms for the quantification of the isoflavone aglycones
265 with the different extraction solvents tested.

266 Figure 1

267 Results obtained by Carrão-Panizzi, Goés-Favoni and Kikuch (2004), who analysed
268 different commercial soy products using 70% ethanol extractor, found values ranging from 65
269 to 168 mg of isoflavones/100 g of soy derivatives. These results were similar to those found
270 in soybean molasses in this study. The extraction with 50% grain alcohol was differentiated
271 from the other solvents presenting higher values of daidzein and genistein (Table 2). The
272 variation in the levels of isoflavones in soybean and its derivatives may change, since they
273 depend on the grain variety, climate, soil and cultivation site. In addition, they may be altered
274 by the temperature and type of processing that foods containing soy derivatives go through
275 (Lee, Gomez, Chang, Wey & Wang 2003).

276 Another important factor to emphasize is the solubility of the isoflavones, the aglycone
277 forms are less soluble in water than the glycosylated and conjugated forms. Studies by Lui,
278 (2004) show that increasing the polarity of the solvent decreased the solubility of the aglycone
279 forms, thus increasing their concentration. This hypothesis was reinforced by the fact that the
280 percentage of the aglycone forms in the supernatants increased with the alcohol concentration,
281 that is, the less polar the solvent, the greater the percentage of aglycones present in the
282 supernatant. However, our studies show opposite results, where the solvent with higher water
283 concentration obtained higher aglycone results.

284

285 *3.2 Microencapsulation of extracts containing isoflavones from soybean molasses*

286

287 The extract obtained with the 50% grain alcohol solvent, for presenting better content
288 of isoflavones and antioxidant activity, was submitted to the microencapsulation process, with
289 different encapsulating agents, at different temperatures of incoming air, as shown in Table 3.

290 Table 3

291 For all input temperatures tested, the outlet temperatures were 90 °C with the exception
292 of T1 (18% maltodextrin DE20) which showed an oscillation at the outlet temperatures of 91,
293 110 and 111 °C. The input temperature is a variable that interferes with the encapsulation
294 efficiency of the process allied with the characteristics of the encapsulating agent (Kissel,
295 Maretschek, Packhauser, Schineiders & Seidel 2006).

296 The encapsulation efficiency of T1 (18% Maltodextrin DE20) differs statistically from
297 the other formulations, thus presenting itself higher in the different temperatures tested,
298 followed by T3 (9% Maltodextrin DE20 and 9% Hi-maize). Studies performed by Silva,
299 Stringheta, Teófilo and Oliveira (2013) when encapsulating jabuticaba bark extract with

300 maltodextrin, using an inlet temperature of 140, 160 and 180 °C, found encapsulation efficiency
301 values of 83.21 and 99%, which were similar to those found in our study for all the treatments
302 analysed.

303 Analysing the treatments with 18% Hi-maize encapsulating agent (T2) in Table 3, the
304 values differed statistically from the other treatments, presenting a mean of 50% retention of
305 the phenolic compounds analysed in this study, when compared to the other treatments that
306 presented retention values of less than 24 and 26% for treatment T1 (18% Maltodextrin DE20)
307 and T3 (18% Maltodextrin DE20 and 18% Hi-maize), respectively. The inlet temperature at
308 130ºC for T2 presented total phenolic values (78.81 ± 0.17 mg GAE/100 g) and total isoflavones
309 (20.44 ± 4.54 mg/100 mL) higher than the other treatments, as well as the treatment that
310 presented higher values of aglycone isoflavones, with values of 2.40 (± 0.43) mg/100 g and
311 1.28 (± 0.28) mg/100 g of daidzein and genistein, respectively (Table 4). The results show that
312 18% hi-maize at 130ºC is the best encapsulating agent in the retention of the phenolic
313 compounds and isoflavones of soybean molasses, differing from other studies showing
314 maltodextrin DE20 as the best encapsulating agent used in the retention of other bioactive
315 compounds.

316 Maltodextrin is one of the most widely used encapsulating agents as a wall drying
317 material for spray drying, due to its high solubility in water (Abadio, Domingues, Borges &
318 Oliveira 2004). Some studies carried out by Murali, Kar, Mohapatra and Kalia (2014) showed
319 that maltodextrin used as wall material for anthocyanin microencapsulates in black carrot juice
320 had the best retention of bioactive compounds when compared to gum arabic and manioc starch.
321 However, the results found in this study showed the 18% hi-maize as the encapsulating agent
322 with higher retention of the studied bioactive compounds in all air temperatures tested. Moser,
323 Souza and Teliz (2017), obtained great results in the retention of phenolic compounds with a
324 mixture of maltodextrin and whey protein isolate (WPI) by microencapsulating grape juice with
325 a 77.9 to 94% retention variation of the phenolic compounds. Analysing the total phenolic
326 compounds and total isoflavones (Table 3), it is possible to observe that there was a degradation
327 of 70 to 40% in all the treatments used. In addition, the hi-maize microcapsule submitted to 130
328 ºC better protected the bioactive compounds when compared to the initial extract containing
329 159.71 mg GAE/100 g and 49.96 (± 5.33) mg/100 g total isoflavones.

330 According to Shahidi and Naczk (2003) and Mahdavi, Jafari, Assadpoor and Dehnad
331 (2016), phenolics and flavonols can form complexes with polysaccharides. Ozdal, Capanogly
332 and Altay (2013) described that some proteins are also capable of forming complexes with
333 phenolic compounds, and that these bonds may be reversible and irreversible, however, the

334 mechanism of the proteins influence on phenolic compounds is not yet known. Therefore, the
335 interactions between the phenolic compounds and the encapsulating agents used may be
336 responsible for the observed retention values.
337 Table 4 shows the results of the antioxidant activity of the microcapsules in relation to
338 the free extract. The T2 treatment differs from the others statistically presenting higher
339 antioxidant activity, followed by microcapsules with 18% maltodextrin DE20 (T1). It is
340 estimated that the decrease in antioxidant activity at all temperatures tested in relation to the
341 free extract occurred due to the loss of retention of the encapsulated compounds. This is because
342 microencapsulation process by spray drying occurs at high temperatures, which makes the loss
343 of the antioxidant activity of the thermosensitive compounds possible, and it can be explained
344 by the high degradation of the isoflavones when being microencapsulated.
345 Table 4
346 Barbosa, Hasimotto, Lajolo and Genovese (2006), studied the levels of isoflavones,
347 phenolic compounds and antioxidant activity in soybean products and found higher antioxidant
348 activity in soybean supplementation (0.78 (± 0.03) µmol BHT/g sample and 5.1 (± 0,1) µmoles
349 of trolox/g of sample), where this same sample presented higher values of phenolics and total
350 isoflavones, in agreement with the results found in this study.
351
352 **4 Conclusion**
353
354 The 50% grain alcohol was the best solvent for extracting the total phenolics and the
355 isoflavones from soybean molasses. This extract showed higher phenolic total isoflavone
356 aglycones, total isoflavones and antioxidant activity.
357 Among the encapsulating agents analysed, the 18% modified hi-maize starch was the
358 best encapsulating agent, presenting an average of 50% retention of the bioactive compounds.
359 Whereas bioactive compounds are extremely sensitive to many factors, the process of
360 microencapsulation by spray drying becomes a challenge. However, it presents economic
361 advantages when compared to other existing encapsulation methods, due to its processing
362 mode, time, and the different storage conditions of the microcapsules, which allows a low cost
363 benefit.
364 Due to the environmental impacts, it is necessary to conduct research involving studies
365 on the extraction of compounds from the by-products and their different applications, making
366 the investment in researches in order to increase the extraction yield of these compounds,
367 together with encapsulating agents suitable to retain the compounds of interest.

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372

373 **Conflict of interest**

374 The authors declare that there are no conflicts of interest.

375

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523 Table 1- Total phenolics, IC_{50} and ORAC of the extracts of the soy molasses obtained by
524 different solvents.
525

Solvents	Total phenolics mg GAE/100 g soy molasses*	IC_{50} mg/g soy molasses*	ORAC µmol Trolox/g soy molasses*
Ethanol 80%	153.09 ^{b} ± 1.36	7.89 ^{a} ± 0.26	211.30 ^{c} ±1.78
Methanol 80%	144.81 ^{c} ± 1.88	6.78 ^{a} ± 0.66	241.85 ^{b} ± 2.67
Grain alcohol 80%	159.42 ^{b} ± 0.0	5.13 ^{b} ± 0.75	266.97 ^{a} ± 2.56
Grain alcohol 50%	244.89 ^{a} ± 11.20	5.03 ^{b} ± 0.25	271.90 ^{a} ±1.80

526 *Results expressed in Mean ± SD (n = 3). Equal lower case letters in the same column do not
527 present significant difference by the Tukey test ($p > 0.05$) for the different solvents with
528 different concentrations. Gallic acid equivalent (GAE).

529 Table 2 - Daidzein, genistein, isoflavones aglycones and total isoflavones from the extract of
530 soybean molasses obtained by different solvents.

Solvents	Daidzein mg/100 g soy molasses*	Genistein mg/100 g soy molasses*	Isoflavones aglycones mg/100 g soy molasses*	Total isoflavones mg/100 g soy molasses*
Ethanol 80%	11.60 ^{c} ± 2.4	3.47^{b} ± 0.1	15.07^{b}	56.84^{c} ± 2.39
Methanol 80%	13.52 ^{b} ± 0.6	2.36^{c} ± 0.0	15.88^{b}	63.31^{b} ± 4.19
Grain alcohol 80%	12.07 ^{c} ± 1.20	3.26 ^{b} ± 0.50	15.33^{b}	46.36^{d} ± 3.80
Grain alcohol 50%	15.38 ^{a} ± 1.50	4.25 ^{a} ± 0.53	19.63^{a}	72.03^{a} ± 1.54

531 *Results expressed in Mean ± SD (n=3). ^{ab} Equal lowercase letters in the same column do not present
532 significant difference by the Tukey test (p> 0.05) for the different solvents with different
533 concentrations.

534 Table 3 - Total phenolics, isoflavones aglycones and total isoflavones from the extract with the
535 best features, microencapsulated with different encapsulating agents at different processing
536 temperatures.

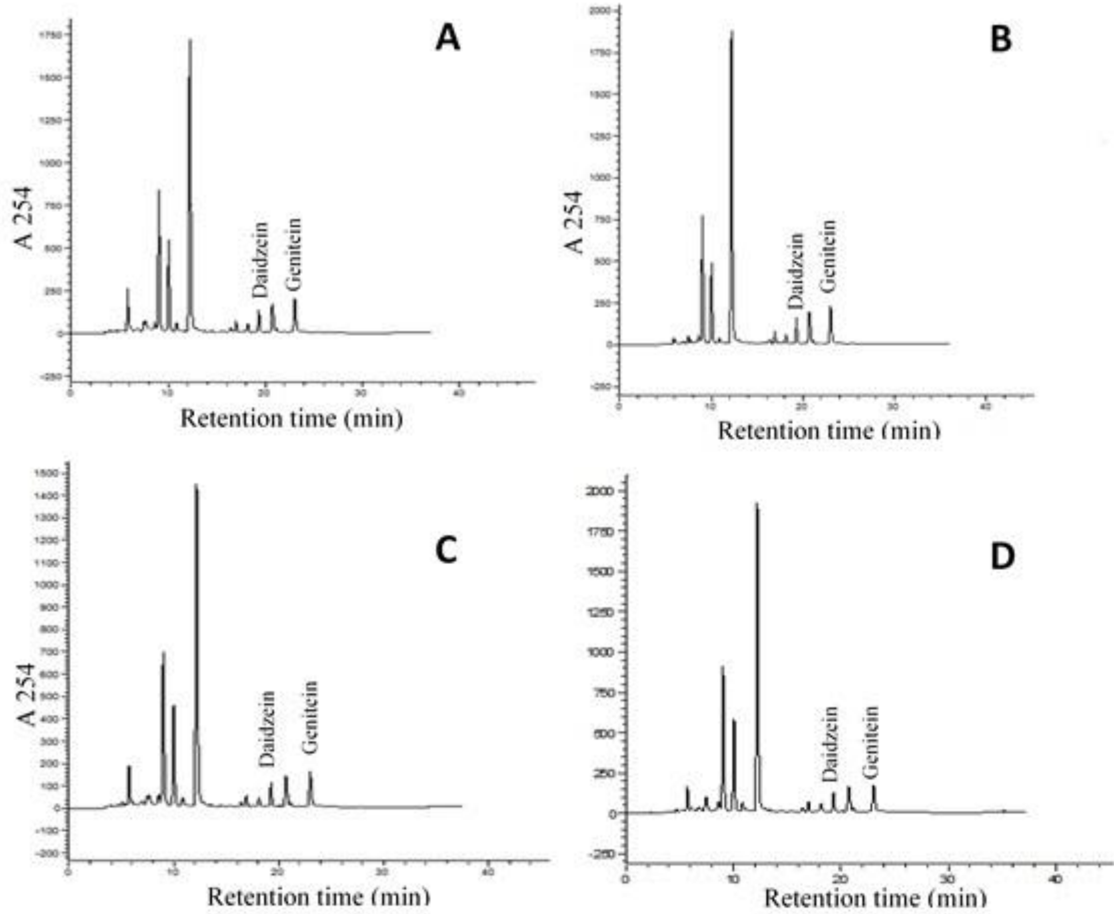
Microcapsules **	Yield (%)	EE (%)	Phenolic* mg GAE/100 g soy molasses	Daidzein* mg/100 g soy molasses	Genistein* mg/100 g soy molasses	Total isoflavones* mg/100 g soy molasses
T1 120ºC	44.30 ^{c}	89.29 ^{a}	40.46 ^{e} ± 0.31	1.11 ^{g} ± 0.15	0.62 ^{f} ± 0.27	11.71 ^{h}± 3.34
T1 130ºC	46.02^{b}	87.49 ^{ab}	39.17 ^{f}± 0.02	0.98 ^{h}± 0.22	0.60 ^{f} ± 0.20	8.14 ^{i}± 1.94
T1 140ºC	39.72^{e}	89.65 ^{a}	36.64 ^{g} ± 0.01	1.70 ^{c} ± 1.20	0.69 ^{f} ± 0.28	15.41 ^{e} ± 4.69
T2 120ºC	37.48^{f}	76.36 ^{c}	76.46^{c} ± 0.19	1.55 ^{e} ± 0.24	0.88 ^{e} ± 0.27	16.62 ^{d} ± 4.68
T2 130ºC	40.23^{d}	76.69 ^{c}	78.81^{b} ± 0.17	2.40^{b} ± 0.43	1.28 ^{b} ± 0.28	20.44 ^{b} ± 4.54
T2 140ºC	37.83^{f}	76.34 ^{c}	78.27^{b} ± 0.09	2.41 ^{b} ± 0.35	0.61 ^{f} ± 0.14	18.77 ^{c} ± 3.04
T3 120ºC	41.71^{d}	88.92 ^{a}	41.24^{e} ± 0.13	1.34 ^{f} ± 0.13	0.83 ^{e} ± 0.31	10.83 ^{f} ± 1.37
T3 130ºC	34.95^{g}	88.78 ^{a}	41.16^{e} ± 0.03	1.61 ^{d} ± 0.27	1.02 ^{c} ± 0.41	13.50 ^{f} ± 3.83
T3 140ºC	53.44^{a}	87.51 ^{ab}	44.34^{d} ± 0.11	1.56 ^{e} ± 0.23	0.96 ^{d} ± 0.41	12.45 ^{g} ± 3.24
Free extract mg/100 g soy molasses	-	-	159.71^{a} ± 0.02	14.92 ^{a} ± 2.76	9.01^{a} ± 2.56	49.96 ^{a} ± 5.33

537 * Results expressed in Mean ± SD (n = 3) ^{ab} Lowercase letters in the same column did not show significant
538 difference by Tukey's test (p> 0.05) (between 120, 130 and 140 °C). Encapsulation efficiency (EE). Gallic acid
539 equivalent (GAE). ** T1= 18% Maltodextrin DE20. T2 = 18% Hi-maize. T3 = 9% Maltodextrin DE20 and 9%
540 Hi-maize.

541 Table 4 - Determination of antioxidant capacity by free radical sequestration of DPPH and
542 oxygen radical absorption capacity (ORAC), the best microencapsulated extract with different
543 encapsulating agents and different inlet air temperatures.

Microcapsules**	IC_{50} (mg/g)*	ORAC (µmol Trolox/g)*
T1 120 ºC	41.05 [d] ± 6.86	25.59 [e] ± 3.40
T1 130 ºC	39.79 [e] ± 3.43	30.83 [d] ± 2.53
T1 140 ºC	36.38 [f] ± 3.56	22.74 [g] ± 2.22
T2 120 ºC	31.05 [g] ± 0.34	90.54 [b] ± 0.97
T2 130 ºC	29.7 [h] ± 0.24	90.35 [b] ± 0.17
T2 140 ºC	28.06 [i] ± 1.63	76.62 [c] ± 1.63
T3 120 ºC	62.99 [b] ± 3.11	23.63 [f] ± 1.40
T3 130 ºC	55.46 [c] ± 4.03	10.40 [i] ± 0.26
T3 140 ºC	61.28 [b] ± 2.15	14.39 [h] ± 0.96
Free extract mg/g	4.73 [a] ± 0.49	194.02 [a] ± 0.96

544 *Results expressed in Mean ± SD (n=3) [ab] Lowercase letters in the same column did not show
545 significant difference by Tukey's test ($p > 0.05$) (between 120, 130 and 140 °C). ** T1= 18%
546 Maltodextrin DE20. T2 = 18% Hi-maize. T3 = 9% Maltodextrin DE20 and 9% Hi-maize.



547

548 Figure 1 - Chromatograms of the isoflavone aglycones with different extraction solvents.

549 Methanol 80% (A); Ethanol 80% (B); Grain alcohol 80% (C); Grain alcohol 50% (D).

4.2 Manuscrito 2

ACCEPTABILITY OF PASTA ADDED OF SOYBEAN MOLASSES MICROCAPSULES[1]

[1]Artigo formatado de acordo com as normas da revista *Food Research International*.

Acceptability of pasta added of soybean molasses microcapsules

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18 **Abstract**

19

20 The aim of this work was to develop fresh pasta with microencapsulated phenolics
21 from soybean molasses, to evaluate the physico-chemical, sensorial and microbiological
22 characteristics as well as the phenolic stability along the pasta storage. The formulations of the
23 pastas were, control (F1), with free extract (F2) and with microcapsules (F3), the analyses of
24 centesimal composition, pH, water activity, colour and sensorial and microbiological analyses
25 were performed. The developed pastas presented themselves within the Standards of Identity
26 and Quality of Pasta. Pastas with free extract and pasta with both raw and cooked microcapsules
27 lost phenolics throughout the storage. However, the cooked pasta with both free extract and
28 those containing microcapsules remained with 25% phenolics until the 14th day, the raw pasta
29 remained with 15% of phenolics until the 21st day of storage. The pastas with the microcapsules
30 showed good acceptability not differing from the control pasta, acceptability index above 70%
31 and purchase intention of 75%. The pasta with pure extract showed less acceptability in
32 attributes, colour, appearance and flavour, acceptability index less than 70% and intention to
33 buy smaller than the others.

34

35 **Keywords:** *Spray-drying*. Stability. Microbiology. Sensory analysis. Bioactive compounds.

36 **1 Introduction**

37

38 Soybean molasses is a by-product obtained from the processing of soybeans in the
39 farinaceous protein production, food used especially for human consumption, becoming a by-
40 product rich in bioactive compounds (Siqueira, 2007). The use of soybean molasses presents
41 economic advantages due to the transformation of the by-product into valuable products,
42 allowing the recapture of its bioactive compounds and commercialization of these compounds
43 by the food and/or pharmaceutical industry (Yang et al., 2009).
44 In view of the great challenge to apply bioactive compounds in food, the
45 microencapsulation process has been broadly studied, and the spray drying method is
46 considered one of the most economical and flexible processes (Suave et al, 2006). The
47 microencapsulation process allows that the core, an encapsulated compound, to be isolated from
48 the external medium, not interacting with the food matrix. This confers greater stability so that
49 the release of the bioactive compounds occurs in the ideal amount and in a suitable period of
50 time (Gouin, 2004), allowing its use in the development of several food products, providing a
51 nutritional increase of these foods (Sobrinho & Farias, 2012). The encapsulating agents are
52 responsible for the coating of bioactive compounds, forming the microcapsules (Azeredo,
53 2005), being the type of encapsulating agent one of the main factors influencing the stability
54 and inhibition of undesirable flavours present in some of these compounds (Suave et al., 2006).
55 The increasing search from the population for healthy foods that, besides their
56 nutritional quality, bring well-being and health benefits, has leveraged a series of studies with
57 this theme (Skrovankova, Sumczynski, Mlcek, Jurikova & Schor, 2015). Considering that, the
58 trend of the current consumer is to use practical food and easy preparation, the consumption of
59 pasta is quite significant in Brazil. From a nutritional and economic point of view, pastas are
60 excellent to be transformed into functional foods and/or enriched by the incorporation of
61 suitable ingredients (Paucar-Menacho et al., 2008, Xavier, 2013). In this context, the aim of this
62 study was to develop pasta with microencapsulated soybean molasses extract and to evaluate
63 the physical-chemical, sensorial, microbiological and shelf life characteristics.

64

65 **2 Materials and methods**

66

67 *2.1 Extraction of Isoflavones, identification of extract and preparation of microcapsules*

68

Soybean molasses, supplied by the industry Selecta (Goiânia, GO), were stored at room temperature until the analyses were carried out. The extraction was carried out using 50% grain alcohol solvent in a ratio of 1:10 sample/solvent (v/v) according to Carrão-Panizzi, Simão and Kikuch (2003) with modifications. The extract was placed under constant agitation for 2 hours at room temperature (shaker 250rpm - Orbital Tecnal Mod. TE140), followed by centrifugation (Centrifuga Serilab MTD III Plus) at 1083 g for 20 min to obtain two fractions: the supernatant and the precipitate. The supernatant was collected and subjected to the drying process by rotaevaporation at 40 °C. An aliquot of the extract was collected for the quantification of isoflavones by HPLC (high performance liquid chromatography). Subsequently 100 mL of the rotaevaporated extract (Buchi model R-3) was submitted to the microencapsulation process, performed on a laboratory scale using mini spray drying (Buchi model B-290) with 1.5 mm feed nozzle with a flow rate of 0.45 L/h. 18% of Hi-maize® (modified starch) was used as wall material, with an inlet air temperature of 130 °C.

2.2 High performance liquid chromatography

The isoflavones were analysed using a high performance liquid chromatography (LC-20A Prominence, Shimadzu, Japan) equipped with a quaternary pump (LC-20AD), manual injector (CTA-20A) and diode arrangement detector (SPD-M20A). The data were processed in the LC solutions program (Version 3, Shimadzu, Columbia, U.S.A). Separation of the compounds was performed on reverse phase column (ODS C18 Microsorb-Mv, 25 mm x 4.6 mm - 5 μm) in gradient elution with flow rate of 0.8 mL / min. Acidified water (0.1% acetic acid) (A) and acidified methanol (0.1% acetic acid) (B) were the solvents used as the mobile phase. The gradient was 0 min, 30% B, in 7 min 40% B, in 15 min 50%, in 25 min 50% B, according to the methodology proposed by Rostagno, Malpa and Barroso (2005).

Prior to the injection, samples were treated for the removal of interferences in polymer resin cartridges (500 mg, 6cc, Dionex™ SolEx™ HRPHS Polymer-Based SPE Cartridge, Thermo Fischer Scientific) for solid phase extraction as described by Kledjus, Vitamvásová and Kubán (1999), with modifications. Prior to injection, all samples were concentrated in rotaevaporator (Buchi R3) and diluted in the initial mobile phase as required. Samples were injected in duplicate using a volume of 50 μl. Samples were filtered on a PTFE syringe filter (0.45 μm, Millipore) prior to injection. The identification of isoflavones was obtained by comparing retention times and spectra in the ultraviolet (UV) range to the visible of the separated compounds with the authentic standards available. Quantification was performed by

103 integrating peak areas at 254 nm. The limits of quantification (LoQ) and detection (LoD) of the
104 method were: LoD = 0.78 and LoQ 2.36 for daidzein and LoD = 0.29 and LoQ = 0.89 for
105 genistein determined by curve data analysis, which were used to quantify the different aglycone
106 isoflavones present in soybean molasses. All compounds with UV-visible spectra similar to that
107 expected for isoflavones and the standards used in this work were considered as compounds
108 derived from this class and totalized for determination of the total isoflavone concentration in
109 the sample. All compounds with UV-visible spectra similar to that expected for isoflavones and
110 the standards used in this work were considered as compounds derived from this class and
111 totalised for determination of the total isoflavone concentration in the sample.
112
113 *2.3 Encapsulation efficiency*
114
115 The microcapsules were dissolved by the method proposed by Robert et al. (2010) with
116 modifications. Where 0.6 g of capsule was weighed and 3 mL of acetonitrile and 3 mL of
117 methanol: acetic acid: water (50:8:42 v/v/v) was added. The mixture was subjected to stirring
118 for 1 min in vortex, afterwards placed in an ultrasonic bath for 60 min, centrifuged at 500rpm/15
119 min the supernatant was collected for analysis of isoflavones, total phenolics and antioxidant
120 activity.
121
122 Surface phenolic compounds (SPC) and encapsulation efficiency (EE) were calculated
123 as described by Robert et al. (2010), according to equations 1 and 2 respectively
124
125 $$SPC\ (\%) = \frac{\text{Surface phenolic compounds}}{theoretical\ content\ of\ phenolic\ compounds}\ x\ 100 \quad \text{(Equation 1)}$$
126
127 $$EE\ (\%) = 100 - SPC(\%) \quad \text{(Equation 2)}$$
128
129 *2.4 Determination of total phenolic compounds of microcapsules and fresh pasta*
130
131 The determination of the phenolic content was set by the Folin-Ciocalteou method
132 described by Singleton, Orthofer & Lamuela-Raventos (1999) with modifications by Roesler
133 (2007). For the colorimetric reaction, 0.4 mL of the extracts previously diluted in the extraction
134 solvent were transferred to the test tubes, after which 2.0 mL of 0.2 N aqueous Folin-Ciocalteau
135 solution (diluted 1:10). After shaking, the tubes were allowed to stand in the absence of light

136 for three minutes. Then, 1.6 mL of sodium carbonate (Na_2CO_3) 7% (m/v) were added and the
137 same incubated for 5 min in a 50 °C water bath. The reading was performed on a
138 spectrophotometer (BEL Photonics 1105) at 760 nm. The results of the total phenolic
139 compounds content were expressed as gallic acid equivalents (mg GAE/100g sample),
140 calculated by means of a calibration Y = 0.0126x - 0.0004, R^2 = 0.9909 which was constructed
141 with the standard of gallic acid at concentrations of 0 to 100 µl/mL. The blank was prepared
142 with the same conditions, but with extract replacement.
143 For determination of the bioactive compounds in the fresh pasta, 5 g of pasta were
144 weighed and 50 mL of 50% grain alcohol were added, with stirring at 700 rpm at room
145 temperature (25 °C). After complete homogenization, the samples were subjected to ultrasonic
146 bath for 10 min, centrifuged at 3000 rpm for 10 min and filtered.
147
148 *2.5 Oxygen radical absorbing capacity (ORAC)*
149
150 The antioxidant capacity was determined by the ORAC (Oxygen Radical Absorbing
151 Capacity) method described by Ou, Hmpsch-Woondill and Prior et al. (2001). This method
152 verifies the efficiency of an antioxidant against a peroxyl radical induced by AAPH at 37 °C.
153 In a 96-well microplate, 25 µL of the diluted extract (200 mg mL^{-1}) was added in phosphate
154 buffer pH 7.4 (75 mmol L^{-1}), followed by incubation on a microplate reader (sense HIDEX,
155 Turku, Finland) for 10 min at 37 °C with 150 µl fluorescein working solution (81 nmol L^{-1}).
156 After incubation, 25 µL of AAPH (152 mmol L^{-1}) were added to form peroxyl radicals.
157 Fluorescence was monitored every minute (excitation and emission wavelengths of 485 and
158 528 nm, respectively) for 121 min at 37 °C. The area under curve (AUC) was calculated by the
159 decay of the fluorescence over time in the presence of an antioxidant agent and the
160 corresponding value of the blank (without antioxidants) was subtracted, obtaining net AUC.
161 The results were compared with a standard Trolox curve (0-96 µmol L^{-1}) and expressed as µmol
162 of Trolox equivalent to 1 g of sample. The AUC was calculated as follows:
163 Where,

164 $$AUC = 1 + {f1}/{f0} + {f2}/{f0} + {f3}/{f0} + \dots {fn}/{f0}$$

165 f 1...fn: fluorescence determined every minute.
166 f0: fluorescence at time zero.
167

168 *2.6 Preparation of fresh pasta*

169

170 The pasta was prepared with the ingredients described in Table 1. After weighing, the
171 ingredients were blended into a homogeneous mass and passed in a cylinder where they were
172 cut into noodle form.
173 Table 1 here
174 The cooking of the pasta was carried out in boiling water in the proportion 1:10, 5 g of
175 pasta in 50 mL of water, sample/solvent (m/v), were added during 3 min. This is a necessary
176 time for the disappearance of the central axis, which was determined by the compression of the
177 cooked pasta sample, every 30 seconds, between two glass slides (AACC, 2000). After the
178 cooking process, the water was discarded and the pasta submitted to the thermal shock process
179 to interrupt the cooking process.

180

181 *2.7 Centesimal composition*

182

183 The centesimal composition of the pasta and soybean molasses followed the
184 methodology described by the Association of Official Analytical Chemists. Carbohydrates were
185 obtained by difference (AOAC, 2002).

186

187 *2.8 pH and water activity*

188

189 The pH was measured using a Digimed® 104 digital potentiometer (Model DM-22)
190 previously calibrated (solutions 4.0 and 7.0). The samples were mixed in distilled water in the
191 proportion 1:10 (AOAC, 2002). For the determination of water activity (Aw) the Aqualab®
192 4TEV apparatus (Decagon Devices, Pullman, WA, USA) was used, the analyses were
193 performed in triplicate.

194

195 *2.9 Stability of phenolics from pasta during storage*

196

197 The pastas were stored refrigerated (4 °C) packed in polyethylene packages. The
198 stabilities of the pasta were evaluated by the loss of the total phenolics, the samples were
199 evaluated at 1, 7, 14, 21 and 28 days.

200

201 *2.10 Color analysis of fresh pasta and microcapsules*
202
203 The colour was analysed through the CIELab system, measuring the parameters *L**, *a**
204 and *b**, where the parameter *L** varies from 0 (black) to 100 (white), indicating a colour
205 variation from black to white. The parameter *a** shows the variation of red (+a*) to green (-a*),
206 while *b** can vary from yellow (+b*) to blue (-b*). Using the Minolta CR-300 colorimeter
207 (Konica Minolta, Osaka, Japan, 1994). The analyses were performed in triplicate with six
208 replicates each.
209
210 *2.11 Microbiological analyses*
211
212 The microbiological evaluation of the fresh pasta followed the methodology established
213 in Normative Instruction No. 62 and the indicated analyses for fresh pasta were carried out by
214 Resolution RDC No. 12 (ANVISA, 2001). 25 g portions of the fresh pasta were homogenized
215 with 225 mL of 0.1% peptone water and the dilutions were used for the microbiological
216 analyses. The coliforms counts were carried out at 35 °C (total), by the most probable number
217 technique, with the use of the culture medium bright green broth. Coliforms at 45 °C in EC
218 broth, positive coagulase *Staphylococcus*, Baird-Parker Agar at 36 °C/48 hours. *Salmonella* sp
219 was subjected to selective enrichment in bright green tetrathionate broth and rappaport
220 vassiliadis (24h /42.5 °C), followed by isolation on plates containing SS agar and Rajhans. The
221 *Clostridium* sulphite reducing counts was performed in SPS medium with the plates incubated
222 in anaerobic jars and count of the total and psychotrophic aerobic mesophilic microorganisms,
223 using Standard agar medium in depth plates for mesophiles and in surface for psychotrophic
224 (37 °C/48 h). *Bacillus cereus* analysis was performed using MYP Agar (Polymixin egg yolk)
225 through surface plaques.
226
227 *2.12 Sensory analysis*
228
229 The sensory analysis was performed after 7 days of the pasta storage in the temperature
230 of 4 °C (± 1). The pasta was cooked for 3 min in salted water and served 10 g of coded pasta
231 with three random digits in disposable white dishes along with a glass of water to clean the taste
232 buds. A total of 100 untrained tasters, randomly recruited at the Centre for Rural Sciences
233 (UFSM), participated in the sensory analysis. The affective test was used to evaluate the
234 acceptance of the products (Monteiro, 1984). The attributes analysed were: colour, odour,

235 flavour, texture, overall appearance, the results were expressed with the 7-point structured
236 hedonic scale, with disliked very much (1), and liked very much (7). It was also evaluated the
237 intention to buy the product with a 5-point attitude scale, ranging from certainly buy (1) and
238 certainly would not buy (5). The samples were distributed in monadic form among the tasters.
239 For the calculation of the acceptability index, the average of the scores obtained from the
240 hedonic scale test was used, knowing that the highest value of the scale (7 = liked very much)
241 is equal to 100% of approval (Equation 3). Results above 70% are considered to have
242 satisfactory repercussions (Monteiro, 1984).
243

244 $$IA\% = \frac{Average\ score\ x\ 100}{7} \quad \text{(Equation 3)}$$

245

246 *2.13 Statistical analysis*

247

248 Data were submitted to analysis of variance (ANOVA), followed by the Tukey test for
249 mean comparisons. The results were considered significant when p <0.05. Statistical analyses
250 were performed in the STATISTICA version 7.0 application (StatSoft Inc, Tulsa - OK, USA).

251

252 **3 Results and discussions**

253

254 *3.1 Isoflavones and phenolics from the free extract and microencapsulated extract of soybean*
255 *molasses.*

256

257 Table 2 shows the values of the isoflavones and phenolics present in 100 mL of the
258 free extract of soybean molasses, corresponding to the amount of extract used during the
259 process of forming the microcapsules, obtaining a quantity of 20 g of microcapsules as final
260 product.

261 Table 2

262 It is observed (Table 2) that the microencapsulation process retained 20% of the
263 compounds present in the extract, justified by the high temperature used by the spray drying
264 microencapsulation method, since the isoflavone concentrations in soy products depend on the
265 conditions of processing, especially the treatment temperature that the food undergoes (Coward,
266 Smith, Kirk & Barners, 1998). The phenolic contents are related to the antioxidant capacity

267 (Gorinstein et al., 2007), because the values of antioxidant activity were reduced after the
268 microencapsulation process of the extract.
269 Although the microencapsulation process presented a high degradation of the bioactive
270 compounds, Hi-maize® as the encapsulating agent has a high encapsulation efficiency, yielding
271 from 20g of capsule to 100mL of encapsulated extract. The encapsulation efficiency defines
272 the amount of substance retained within the microcapsule and depends from, among other
273 factors, the air inlet temperature, the encapsulating agent, as well as the affinity between the
274 encapsulating agent and the substance to be encapsulated (Kissel, Maretschek, Packhauser &
275 Schineiders, 2006).
276
277 *3.2 Centesimal composition of fresh pasta*
278
279 Data on the centesimal composition of the pasta and soybean molasses are presented in
280 Table 3.
281 Table 3
282 It can be observed in Table 3 that there was no significant difference in moisture content
283 among treatments. The values are in compliance with the RDC N ° 93 Standards of Identity and
284 Quality of Pasta (ANVISA, 2000) which stipulate a maximum of 35.0% (g/100 g) of moisture
285 for fresh pasta. The small variations in the centesimal composition of the pastas are due to the
286 composition of the soybean molasses, being that the F2 differs from the others with higher
287 protein, fiber and ethereal extract contents, justifiable by the extract being in its free form. The
288 oscillation of the centesimal composition of soybean molasses is considered normal, due to its
289 form of production that comes from the concentration of the proteins present in the soybean
290 meal.
291
292 *3.3 Stability of phenolics from fresh pasta*
293
294 Table 4
295 According to Table 4, the pastas did not present a significant difference in pH, however,
296 from the seventh day, the pH gradually decreased. The water activity remained stable
297 throughout the storage.
298 The pastas with free extract and pastas with both raw and cooked microcapsules lost
299 phenolics throughout the storage. However, the cooked pasta with both free extract and those
300 containing microcapsules remained with 25% phenolics until the 14th day, the raw pasta

301 remained with 15% of phenolics until the 21st day of storage. Cooking probably affected the
302 wall material of the microcapsules, as well as the leaching process that occurs in the cooking
303 process of pastas.
304 At the end of the 21 days of storage, the crude pastas added with free extract (F2)
305 remained with 13.65% of phenolics; however, the pastas with the microcapsules retained 17.9%
306 of the compounds, showing that the raw pastas with the microcapsules had a longer shelf life
307 in terms of phenolics. It was not carried out an analysis of the time of 28 days because the pastas
308 are consumed cooked and those at 21 days no longer contained phenolic.
309
310 *3.4 Colour parameters of fresh pasta and microcapsule*
311
312 Table 5
313 In relation to the variable L*, values ranged from 78.49 to 57.69 in the different
314 formulations (Table 5). Throughout the storage, the values of L* were decreasing, probably by
315 the oxidation process. It is also observed that the luminosity decreased with addition of the pure
316 extract of soybean molasses (F2) and with the addition of the microencapsulated extract (F3),
317 which is a negative aspect, knowing that low values are unfavourable to the attractiveness of
318 the pastas. It is suggested that the darker coloration of the pasta occurs due to the darker natural
319 colour of soybean molasses as well as of the encapsulating agent (hi-maize). The
320 microencapsulated extract has a luminosity of 52.63.
321 The variable a* ranged from 7.18 to 10.01. The results differed significantly between
322 the different formulations during the same storage period, and the formulations F2 and F3,
323 which received addition of pure extract and microencapsulated extract of soybean molasses,
324 showed an increase in the a* variable.
325 The averages of variable b* varied from 21.18 to 23.43, formulations F2 and F3 differed
326 significantly only from the control, throughout the refrigerated storage. The values found in the
327 different formulations in this study are below the values found by Paucar-Menacho et al. (2008),
328 who found values greater than 28.41 of variable b* in different concentrations of soy protein
329 isolate and polydextrose in their formulations of functional fresh pasta.
330
331 *3.5 Microbiological analyses of pasta*
332
333 Table 6

334 According to the National Health Surveillance Agency (ANVISA), resolution 12
335 (BRAZIL, 2001) that establishes the technical regulation of microbiological standards for food,
336 the fresh pasta analysed in this study is within the required standards where the maximum
337 values for coliforms at 45 °C/ g is 102 CFU/ g. For positive coagulase *Staphylococcus* $5x10^3$
338 CFU/ g, for *Bacillus cereus* is $5x10^2$ CFU/ g and for *Salmonella* sp, absence in 25 g. The
339 coliform counts at 35 °C for the control formulation was $9x10^2$ and the formulation containing
340 the microcapsules was $9x10^1$ NmP/ mL, for the formulation containing the pure extract was
341 $4x10^3$ NmP/ mL, all values were lower than the values allowed by the legislation. The coliform
342 counts at 45 °C in the different fresh pasta formulations were below 10^1 CFU/ g.
343 Table 7
344 By observing the mesophilic aerobic microorganisms count (Table 7), it is verified that
345 the counts increase throughout the refrigerated storage in all the treatments, which is normal,
346 since the refrigeration slows, but does not inhibit the microbial growth.
347 In the psychotrophic bacteria count, the formulation with the free extract showed values
348 $<1 \times 10^1$ Log CFU/ g throughout the storage, differing statistically from the formulations F1
349 and F3 on day 7 and 14 which showed a gradual increase of the count. Studies by Mantovani,
350 (2013), when evaluating the antimicrobial activity of isoflavone extracts from soybean
351 molasses, bioconverted in aglycones, also observed the antimicrobial effect of the extract.
352 According to Cook and Samman (1996), the aglycone isoflavones have hydroxyl grouping
353 responsible for the antimicrobial effect.
354
355 *3.6 Sensory analysis*
356
357 Table 8
358 Table 8 shows that the acceptability of the pastas with the microcapsules did not differ
359 from the control pasta, the averages for the attributes taste, texture and appearance were
360 between "liked" and "really liked", showing good acceptability. However, the pasta with the
361 extract (F2) differs from the others with less acceptance in the attributes of colour, flavour and
362 appearance, due to the characteristics of the soybean molasses extract being dark, having a
363 strong flavour and a darker-looking pasta. The results of Table 9 show that the control pastas
364 and the pastas containing the microcapsules have an acceptability of more than 70% in all
365 attributes analysed.
366 Table 9

367 The intention of purchase test showed that: 77% of the testers would buy the control
368 formulation, 69% would buy the formulation containing free extract and 75% would buy the
369 formulation containing microencapsulated extract (Figure 1).
370 Figure 1
371
372 **4 Conclusion**
373
374 The pastas presented within the Standards of Identity and Quality of Pasta, throughout
375 the storage were darker in all formulations.
376 Pastas with free extract and pasta with both raw and cooked microcapsules lost
377 phenolics throughout the storage. However, the cooked pasta with both free extract and the
378 containing microcapsules remained with 25% of phenolic until the 14th day, the raw pasta
379 remained with 15% phenolic until the 21st day of storage.
380 The developed pastas are in accordance with DRC No. 12, the psychrotrophs counts
381 increased along storage in the control pasta and in pasta with the microcapsules. The pure
382 extract acted as an antimicrobial enabling the pasta shelf life to increase.
383 The pastas with microcapsules presented good acceptability not differing from the
384 control pasta, acceptability index above 70% and purchase intention of 75%.
385
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390
391 **Compliance with Ethical Standards Conflict of Interest**
392 The authors declare that they have no conflict of interest.
393
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477 Yang, Q., Peng, X., Yang, L., Han, J., Yang, Y., & Wu, G. (2009). The utilisation of vinegar 478 waste. *Journal Shanxi Agricultural Science***,** 37, 44–46.

479 Table 1 - Ingredients used in fresh pasta formulations.

Fresh pasta			
Ingredients	Control	Free extract	Microencapsulated extract
Water (mL)	130	90	140
Wheat Flour Type 1 (g)	426	426	386
Egg (g)	47	47	47
Salt (g)	8	8	8
Extract (mL)	-	40	-
Microcapsules (g)	-	-	40

480 Control (F1); Free extract (F2) 40 mL of free extract, corresponds to 66 mg of total phenolics;
481 Microencapsulated extract (F3) 40g of capsules corresponds to 60.56 mg of total phenolics.

482 Table 2 - Isoflavones, phenolics, antioxidant activity and encapsulation efficiency of pure
483 extract and microencapsulated extract of soybean molasses.

	Daidzein	Genistein	Total isoflavones	Total phenolics	ORAC (µmol Trolox/mL)	EE%*
Free extract mg GAE*/100mL	19.83^a ± 0.71	5.41^a ± 0.01	92.95^a ± 0.97	167.49^a ±0.02	191.02^a ± 0.96	-
18% Hi-maize® mg GAE*/ 20g	3.21^b ± 0.12	0.61^b ± 0.02	19.03^b ± 0.85	30.28^b ±0.03	90.03^b ± 0.17	76.69

484 Results expressed in Mean ± SD (n=3) ^{ab} Equal lowercase letters in the same column did not present
485 significant difference by the Tukey test ($p < 0.05$). *Gallic acid Equivalent (GAE). Yield (R). Encapsulation
486 efficiency (EE).

487 Table 3 - Centesimal composition of control pasta, with extract, microcapsules and soybean
488 molasses.

Constituents g (%)	Fresh pasta			Soybean molasses
	F1	F2	F3	
Moisture	33.24[a]	34.38[a]	34.71[a]	22.24
Ashes	1.64[b]	1.68[b]	1.70[b]	2.23
Crude protein	8.60[b]	9.21[a]	8.35[b]	7.09
Ethereal extract	0.58[c]	0.74[b]	0.54[c]	14.00
Total Fiber	0.30[b]	0.43[a]	0.28[c]	0.37
Carbohydrates	55.64[a]	53.56[d]	53.90[c]	54.07

489 Results expressed in Mean ± SD (n=3) [ab] Equal lowercase letters in the same row did not present
490 significant difference by the Tukey test ($p < 0.05$). F1 - Formulation control; F2 - Formulation
491 containing pure extract; F3 - formulation containing microencapsulated extract.

492 Table 4 - pH values, water activity, total phenolics of the raw and cooked pasta, during 21 days
493 of refrigerated storage at 4° C (± 1).

Fresh pasta		Raw			Cooked
		pH	Aw	Total phenolics mg GAE/100 g	Total phenolics mg GAE/100 g
1st Day	F1	6.46^{A} ±0.00	0.96^{A} ±0.00	1,02^{Ga} ±0.00	0,85^{Ga} ±0.01
	F2	6.49^{A}±0.01	0.97^{A} ±0.00	60,28^{Aa} ±0.02	35.09^{Bb} ±0.02
	F3	6.45^{A} ±0.00	0.96^{A} ±0.00	61,92^{Aa} ±0.11	42,66A^{Ab} ±0.01
7th Day	F1	6.18^{D} ±0.01	0.97^{A} ±0.00	0,91^{Ha} ±0.01	0,75^{Db} ±0.02
	F2	6.23^{C} ±0.02	0.96^{A} ±0.00	55,88^{Ba} ±0.00	22,18^{Db} ±0.01
	F3	6.33^{B} ±0.01	0.96^{A} ±0.00	61,60^{Aa} ±0.01	24,67^{Cb} ±0.01
14th Day	F1	6.12^{E} ±0.02	0.96^{A} ±0.00	0,86^{Ia} ±0.00	0,43^{Hb} ±0.01
	F2	6.12^{E} ±0.01	0.96^{A} ±0.00	42,23^{Da} ±0.00	8,74^{Fb} ±0.02
	F3	6.10^{E} ±0.01	0.96^{A} ±0.00	45,14^{Ca} ±0.01	10,49^{Eb} ±0.02
21st Day	F1	6.05^{F} ±0.01	0.96^{A} ±0.00	0,45^{Ja} ±0.02	0,02 ^{Kb} ±0.00
	F2	6.05^{F} ±0.02	0.96^{A} ±0.00	8,23^{Fa} ±0.02	0,04^{Jb} ±0.00
	F3	6.06^{F} ±0.01	0.96^{A} ±0.00	11,08^{Ea} ±0.01	0,07^{Ib} ±0.00

494 Results expressed in Mean ± SD (n=3) ^{AB} Equal upper case letters in the same column did not present
495 significant difference by the Tukey test ($p < 0.05$) between the different formulations. ^{ab} Equal
496 lowercase letters on the same line do not show significant difference by the Tukey test ($p < 0.05$)
497 between the same formulations on the same storage day. Formulation control (F1); Formulation
498 containing free extract (F2); Formulation containing microencapsulated extract (F3).

499 Table 5 **-** Instrumental colour parameters of the fresh pasta after application of the pure extract
500 and microencapsulated extract of soybean molasses for 21 days of storage at 4 °C (± 1).

Storage	Fresh pasta Formulations	Colour parameters		
		L*	a*	b*
1st Day	F1	$78.49^{Aa} \pm 1.10$	$7.18^{Ca} \pm 0.51$	$21.53^{Ba} \pm 0.83$
	F2	$71.87^{Ca} \pm 0.44$	$8.09^{Ba} \pm 0.11$	$23.43^{Aa} \pm 0.13$
	F3	$73.21^{Ba} \pm 1.11$	$10.01^{Aa} \pm 0.26$	$23.28^{Aa} \pm 0.33$
7th Day	F1	$74.10^{Ab} \pm 0.72$	$6.88^{Ba} \pm 0.18$	$18.45^{Bb} \pm 0.16$
	F2	$69.39^{Cb} \pm 0.70$	$8.59^{Aa} \pm 0.23$	$21.99^{Ab} \pm 0.38$
	F3	$70.49^{Bb} \pm 0.51$	$8.97^{Aa} \pm 0.13$	$22.05^{Ab} \pm 0.63$
14th Day	F1	$72.94^{Ac} \pm 0.22$	$7.40^{Ca} \pm 0.21$	$18.20^{Bb} \pm 0.19$
	F2	$62.94^{Bc} \pm 1.83$	$8.99^{Ba} \pm 0.03$	$22.29^{Aab} \pm 0.19$
	F3	$60.83^{Cc} \pm 1.07$	$9.66^{Aa} \pm 0.19$	$22.83^{Aab} \pm 0.78$
21st Day	F1	$70.08^{Ad} \pm 0.21$	$8.09^{Ca} \pm 0.19$	$18.09^{Bb} \pm 0.18$
	F2	$60.23^{Bd} \pm 0,23$	$9.13^{Ba} \pm 0.05$	$21.18^{Abc} \pm 0.18$
	F3	$57.69^{Cd} \pm 0,88$	$9.55^{Aa} \pm 0.17$	$22.27^{Aab} \pm 0.73$
Microencapsulated extract		$52.63^{D} \pm 0.12^{d}$	$3.10^{D} \pm 0.33$	$9.37^{C} \pm 0.14$

501 Results expressed in Mean ± SD (n=3) ^{AB} Equal upper case letters in the same column did not show
502 significant difference by Tukey's test ($p < 0.05$) between the different formulations on the same
503 storage day. ^{ab} Equal lowercase letters in the same column do not present significant difference by
504 the Tukey test ($p < 0.05$) between the same formulations throughout the storage. Formulation
505 Control (F1); Formulation containing free extract (F2); Formulation containing microencapsulated
506 extract (F3). *L = Luminosity; a = red to green; b = yellow to blue.

507 Table 6 - Microbiological count (CFU/g) of the different formulations of fresh pasta.

508 Microbiological count (CFU/g) of the different formulations of fresh pasta.

CFU/g	F1	F2	F3
Bacillus Cereus	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$
Coliforms 45 °C	< 3.0 NmP/g	< 3.0 NmP/g	< 3.0 NmP/g
Clostridium	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$	$< 1.0 \times 10^1$
Salmonella sp/25 g	Absent	Absent	Absent
Coagulase positive *Staphlylococcus*	$< 1.0 \times 10^2$	$< 1.0 \times 10^2$	$< 1.0 \times 10^2$

509 Formulation control (F1); Formulation containing free extract (F2); Formulation containing
510 microencapsulated extract (F3). CFU - colony forming unit. NmP - most likely number.

Table 7 - Microbiological stability of fresh pasta.

Total Mesophilic Aerobes (Log CFU/ g)			
Days	F1	F2	F3
1	1.6 x $10^{3\text{Ba}}$	8 x $10^{2\text{Bc}}$	1.25 x $10^{3\text{Ba}}$
7	6.9 x $10^{2\text{Ca}}$	3.9 x $10^{2\text{Bb}}$	3.4 x $10^{2\text{Cb}}$
14	>5 x $10^{5\text{Ab}}$	2.2 x $10^{3\text{Aa}}$	>5 x $10^{5\text{Ab}}$
Psychotropic Bacteria (Log CFU/g)			
Days	F1	F2	F3
1	< 1 x $10^{1\text{Ca}}$	< 1 x $10^{1\text{Aa}}$	< 1 x $10^{1\text{Ca}}$
7	1.3 x $10^{2\text{Ba}}$	< 1 x $10^{1\text{Ac}}$	6 x $10^{1\text{Bb}}$
14	8 x $10^{3\text{Aa}}$	< 1 x $10^{1\text{Ac}}$	2.9 x $10^{3\text{Ab}}$

Results expressed in Mean ± SD (n=3)^{AB} Equal upper case letters in the same column do not present significant difference by the Tukey test ($p <0.05$) between the same formulations throughout the storage. ^{ab} Equal lowercase letters in the same line did not present significant difference by the Tukey test ($p <0.05$) between the different formulations on the same storage day. Formulation control (F1); Formulation containing free extract (F2); Formulation containing microencapsulated extract (F3).

518 Table 8 **-** Acceptance test for different formulations of fresh pasta.

Atributes	Fresh pastas		
	F1	F2	F3
Colour	$4.92^{a} \pm 0.92$	$4.49^{b} \pm 1.03$	$5.02^{a} \pm 0.98$
Odour	$4.93^{a} \pm 1.02$	$4.87^{a} \pm 1.06$	$4.91^{a} \pm 0.96$
Flavour	$5.18^{a} \pm 1.38$	$4.67^{b} \pm 1.12$	$5.07^{a} \pm 1.02$
Texture	$5.22^{a} \pm 1.13$	$5.16^{a} \pm 1.26$	$5.29^{a} \pm 1.01$
Appearance	$5.29^{a} \pm 1.08$	$4.73^{b} \pm 0.89$	$5.16^{a} \pm 1.02$

519 Results expressed in Mean ± SD (n=3) [ab] Equal lowercase letters in the same row did not present
520 significant difference by the Tukey test ($p < 0.05$). Formulation control (F1); Formulation
521 containing free extract (F2); Formulation containing microencapsulated extract (F3). Scores: 1 =
522 I really disliked; 2 = I disliked it a lot; 3 = disliked; 4 = indifferent; 5 = liked; 6 = I liked it a lot;
523 7 = I really liked.

Table 9 - Index of acceptability (%) of fresh pasta.

Attributes	Fresh pasta		
	F1	F2	F3
Colour	70.28^{b}	64.14^{c}	71.71^{a}
Odour	70.42^{a}	69.57^{b}	70.14^{a}
Flavour	74.00^{a}	66.71^{c}	72.42^{b}
Texture	74.57^{b}	73.71^{c}	75.57^{a}
Appearance	75.57^{a}	67.57^{b}	73.71^{b}

Results expressed in Mean ± SD (n=3)^{ab} Equal lowercase letters in the same row did not present significant difference by the Tukey test ($p < 0.05$). Formulation control (F1); Formulation containing free extract (F2); Formulation containing microencapsulated extract (F3).

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529 Figure 1 - Test of intention to buy fresh pasta. *Formulation control (F1); Formulation*
530 *containing free extract (F2); Formulation containing microencapsulated extract (F3).*

5 CONCLUSÃO GERAL

O álcool de cereais a 50% foi o melhor solvente para extrair as isoflavonas, este extrato apresentou maior rendimento de isoflavonas agliconas, isoflavonas totais, fenólicos e atividade antioxidante.

O amido modificado hi-maize utilizado no método de *spray drying* com temperatura de entrada de ar de 130º C foi o melhor agente encapsulante, apresentando menor degradação de isoflavonas totais, isoflavonas agliconas e fenólicos totais, este extrato também mostrou melhor atividade antioxidante, sendo a atividade antioxidante no extrato maior que nas microcápsulas em ambos os métodos.

As massas contento as microcápsulas foram capazes de manter meior teor de compostos fenólicos num período de 14 dias de armazenamento refrigerado, diferenciando-se estatisticamente das demais formulações. Os resultados também mostraram que as massas cozidas mostraram uma degradação significativa dos compostos bioativos em relação as in natura ao longo do armazenamento e em todas as formulações, a partir do décimo quarto dia o cozimento das massas degradou os compostos em todas as formulações.

O pH apresentou pequena variação no sétimo dia e a atividade de água manteve-se igual ao longo do armazenamento. As massas também mostraram-se mais escuras em todos os tratamentos ao longo do armazenamento. As massas com as microcápsulas não diferenciaram da massa controle, apresentando boa aceitação com índice de aceitabilidade acima de 70% e intenção de compra de 75%. A massa com extrato mostrou menor aceitabilidade nos atributos, cor, aparência e sabor, com índice de aceitabilidade menor que 70% e intenção de compra menor que as demais.

As massas desenvolvidas apresentaram-se dentro dos Padrões de Identidade e Qualidade de Massas Alimentícias, as massas desenvolvidas estão de acordo com a resolução n°12 que estabelece o regulamento técnico de padrões microbiológicos para alimentos, as contagens de psicrotróficos aumentou ao longo do armazenamento na massa controle e na massa com as microcápsulas. Entretanto a adição de extrato puro agiu como um antimicrobiano aumentando a vida de prateleira dessa massa.

A utilização do resíduo melaço de soja, apresenta vantagens econômicas devido a possibilidade de comercialização das isoflavonas, e a possibilidade de adiciona-las em alimentos e fim de agregar valor a alimentos já disponíveis no mercado. No entanto, ainda são necessários mais estudos frente a outros métodos de encapsulação e agentes encapsulantes para

esse composto bioativo, devido a elevada porcentagem de degradação desse composto em relação ao seu extrato inicial.

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ANEXOS

Anexo 1 - Resultados de fenólicos totais nas extrações exaustivas dos diferentes solventes analisados na mesma concentração.

Extração	Álcool de cereais 80%*	Etanol 80%*	Metanol 80%*
Extração 2h	143,96 ^{Aa} ±0,0	132,00 ^{Ba} ±1,36	119,01 ^{Ca} ±1,88
Exaustiva 4h	16,42 ^{Cb} ±1,03	21,09 ^{Bb} ±2,11	25,80 ^{Ab} ±0,13
Exaustiva 6h	5,91 ^{Ac} ±0,17	5,82 ^{Ac} ±0,04	1,45 ^{Bc} ±0,06

*Resultados são expressos em Média ± DP (n=3). ^{a,b} Letras minúsculas iguais na mesma coluna não apresentam diferença significativa pelo teste de Tukey (p<0,05) para o mesmo solvente na extração exaustiva. ^{AB} letras maiúsculas iguais na mesma linha não apresentam diferença significativa pelo teste de Tukey (p>0,05), para os diferentes solventes em diferentes concentrações, na mesma extração exaustiva. Resultados expressos mg GAE/100g. Equivalente de ácido gálico(GAE).

Anexo 2 – Resultados de fenólicos totais e atividade antioxidante pelo método DPPH, dos extratos de melaço de soja com mesmo solvente em diferentes concentrações.

	Álcool de Cereais			
	50%	**60%**	**70%**	**80%**
Fenólicos totais mg GAE/100g melaço de soja	244,89^{A} ± 11,20	229,89^{B} ± 9,92	244,34^{A} ± 11,91	232,02^{B} ± 11,91
IC_{50} mg/g de melaço de soja	5,06^{B} ± 0,25	9,07^{A} ± 0,81	5,96^{C} ± 0,45	5,13^{B} ± 0,75

Resultados são expressos em Média ± DP (n=3). ^{ABb} Letras iguais na mesma linha não apresentam diferença significativa pelo teste de Tukey (p>0,05) para o mesmo solvente em diferentes concentrações. Equivalente de ácido gálico (GAE).

Anexo 3 – Cromatogramas da separação e quantificação das isoflavonas do melaço de soja por cromatografia Líquida de Alta Eficiência (CLAE).

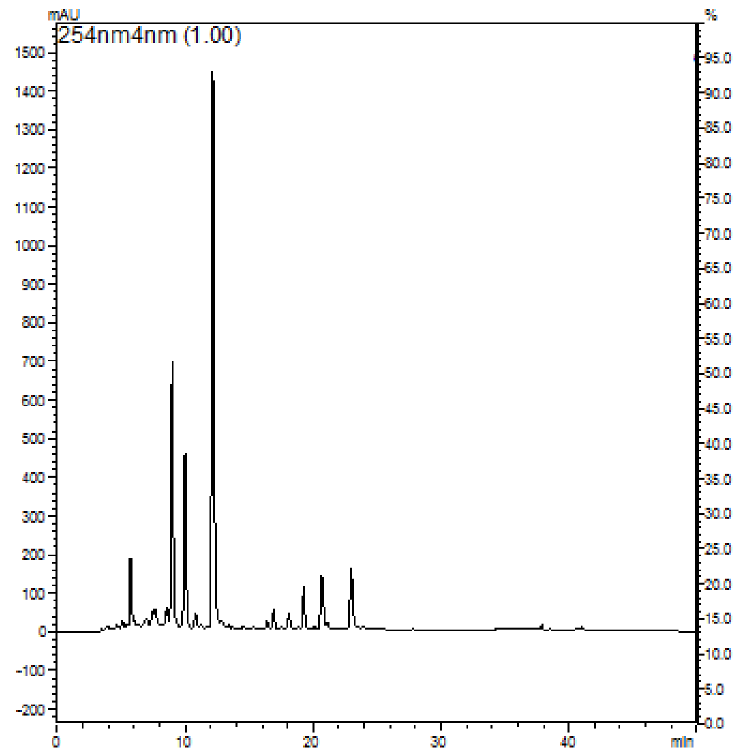

Figura 1 – Cromatograma isoflavonas com álcool de cereais 80%.

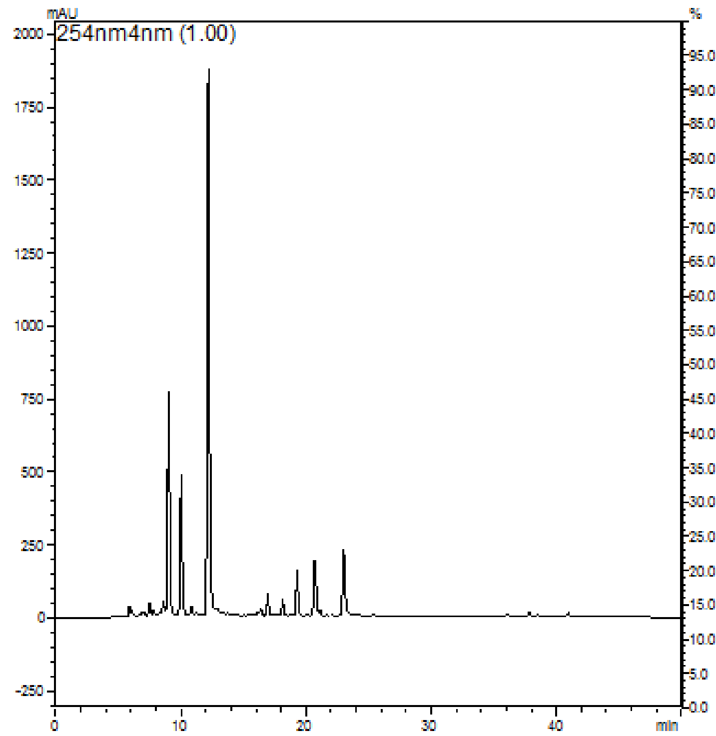

Figura 2 – Cromatograma isoflavonas com solvente etanol 80%

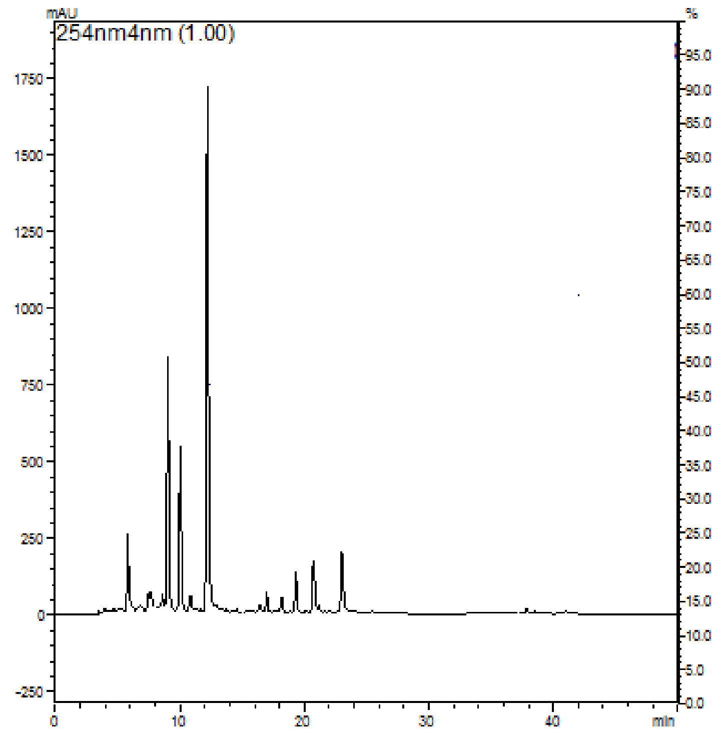

Figura 3 – Cromatograma isoflavonas com solvente metanol 80%

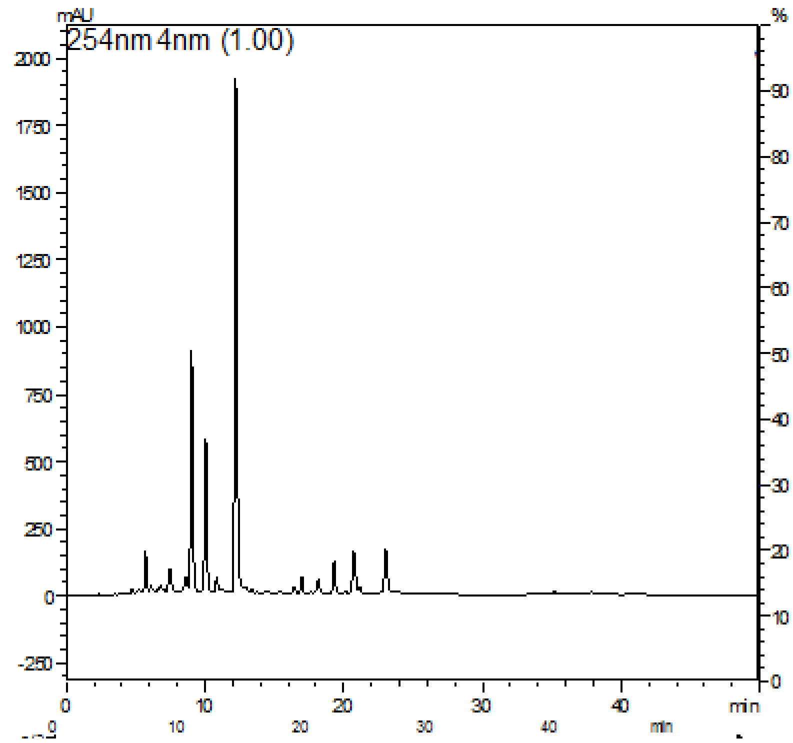

Figura 4 – Cromatograma isoflavonas com álcool de ceraos 50%

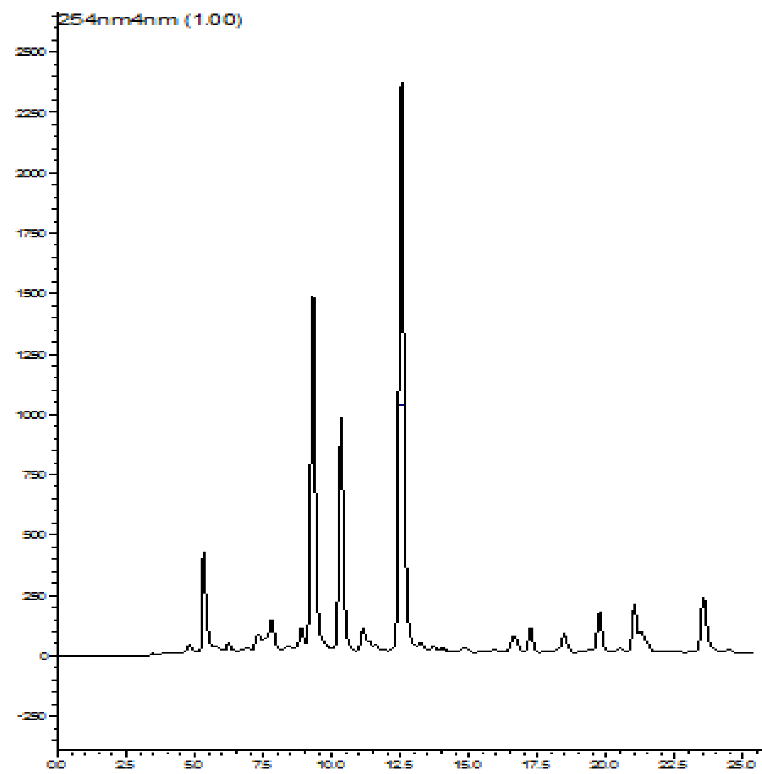

Figura 5 - Cromatograma isoflavona álcool de cereais 80% com cartuchos resina polimérica (500mg, 6cc, Dionex™ SolEx™ HRPHS Polymer-Based SPE Cartridge, Thermo Fischer Scientific)

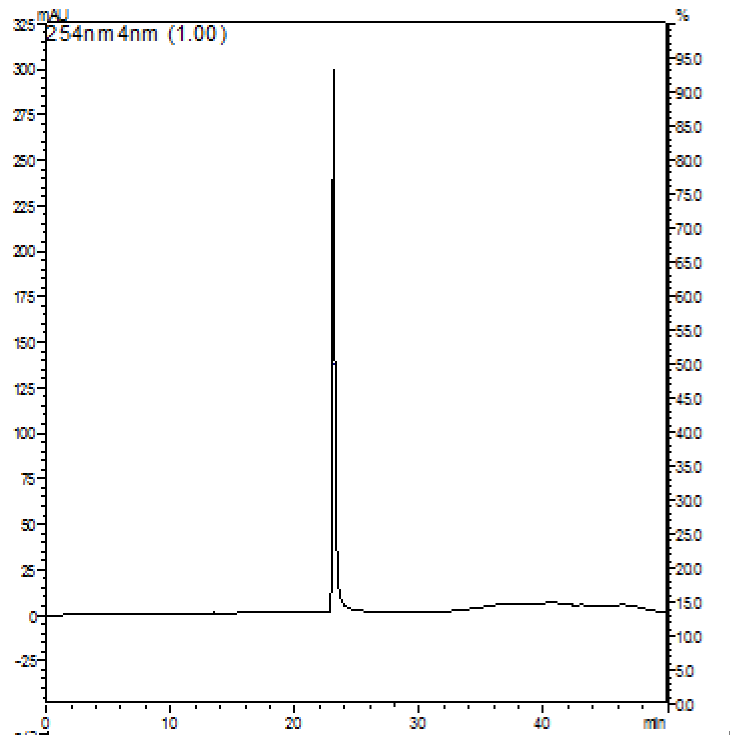

Figura 6 – Cromatograma da curva padrão genisteína.

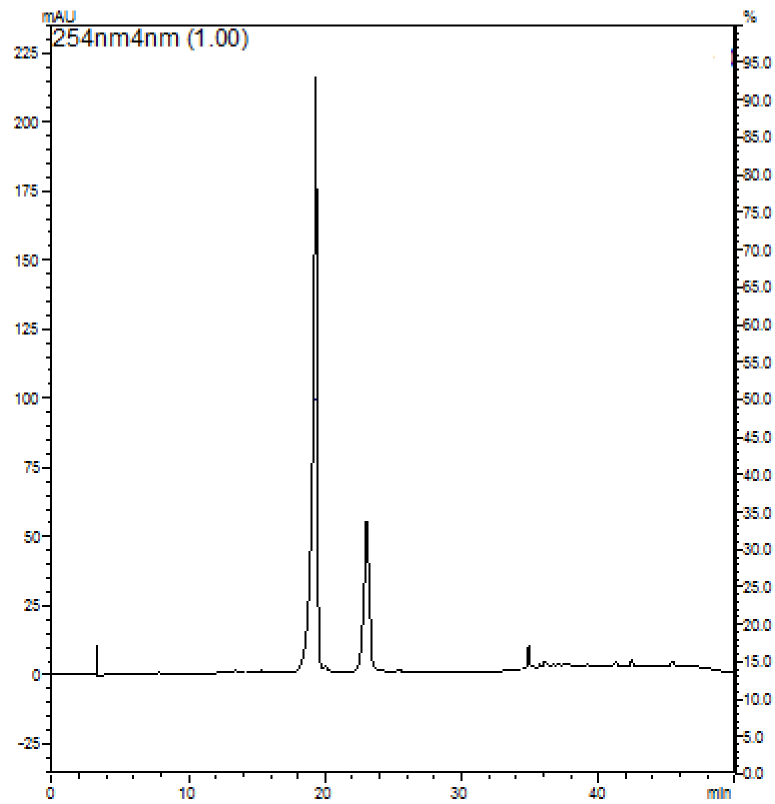

Figura 7 – Cromatograma da curva padrão daidzeína

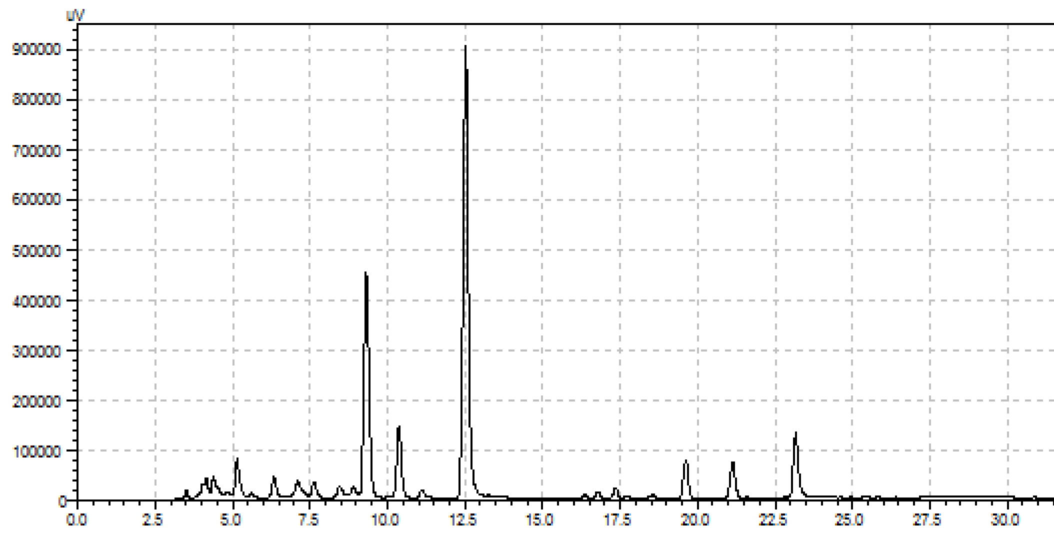

Figura 8 – Cromatograma isoflavonas microencapsulação T1 120°C

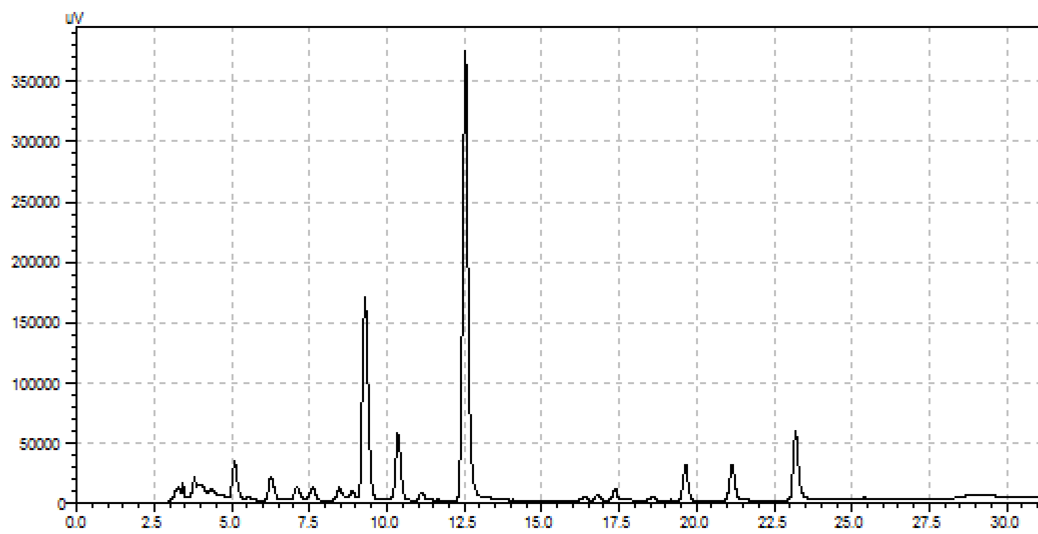

Figura 9 – Cromatograma isoflavonas microencapsulação T1 130°C

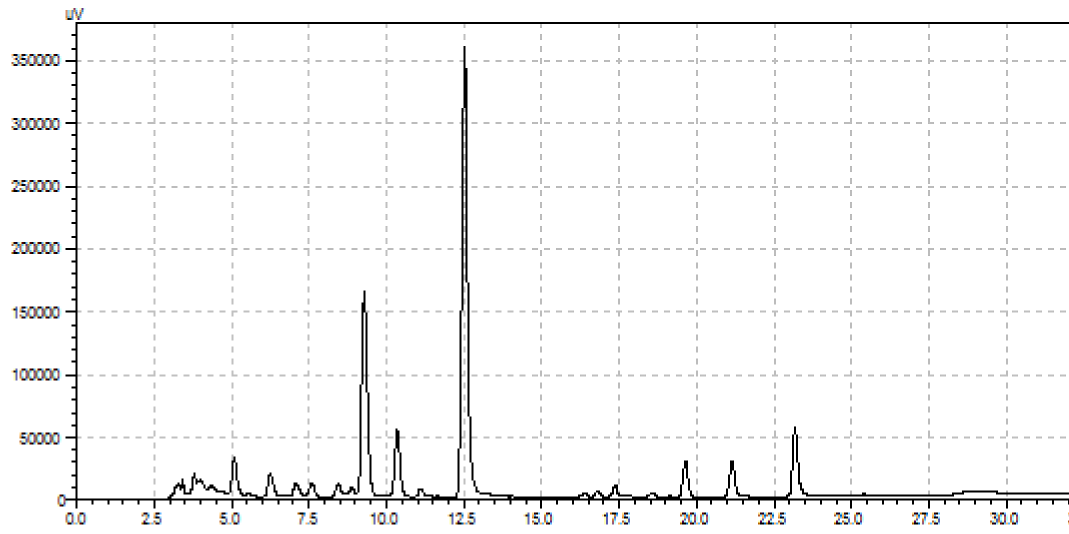

Figura 10 – Cromatograma isoflavonas microencapsulação T1 140°C

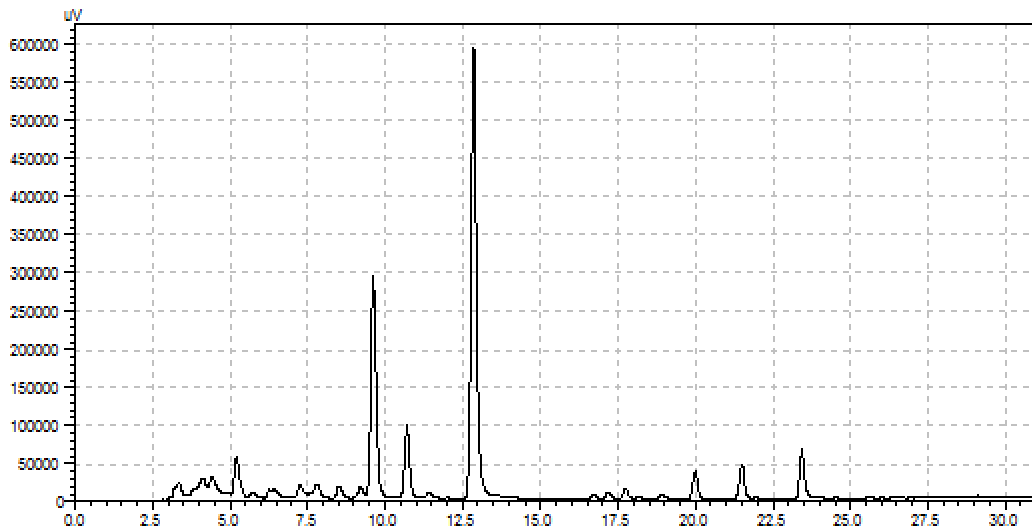

Figura 11 – Cromatograma isoflavonas microencapsulação T2 120°C

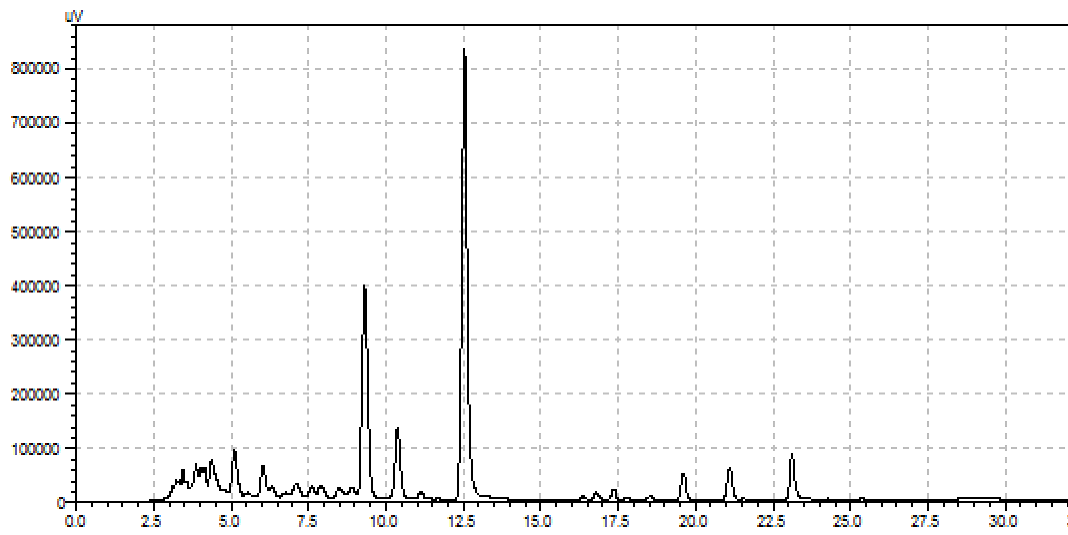

Figura 12 – Cromatograma isoflavonas microencapsulação T2 130°C

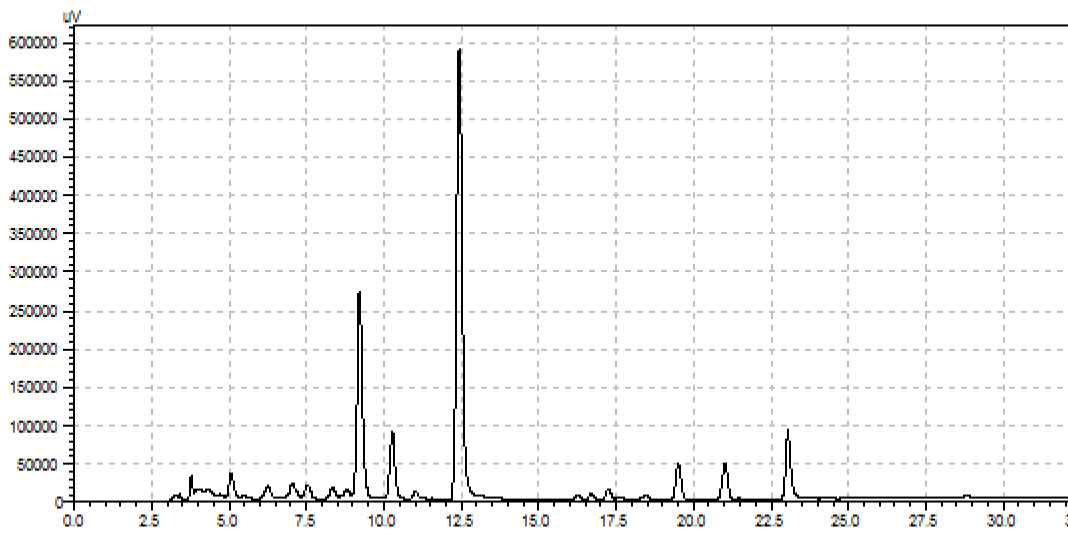

Figura 13 – Cromatograma isoflavonas microencapsulação T2 140°C

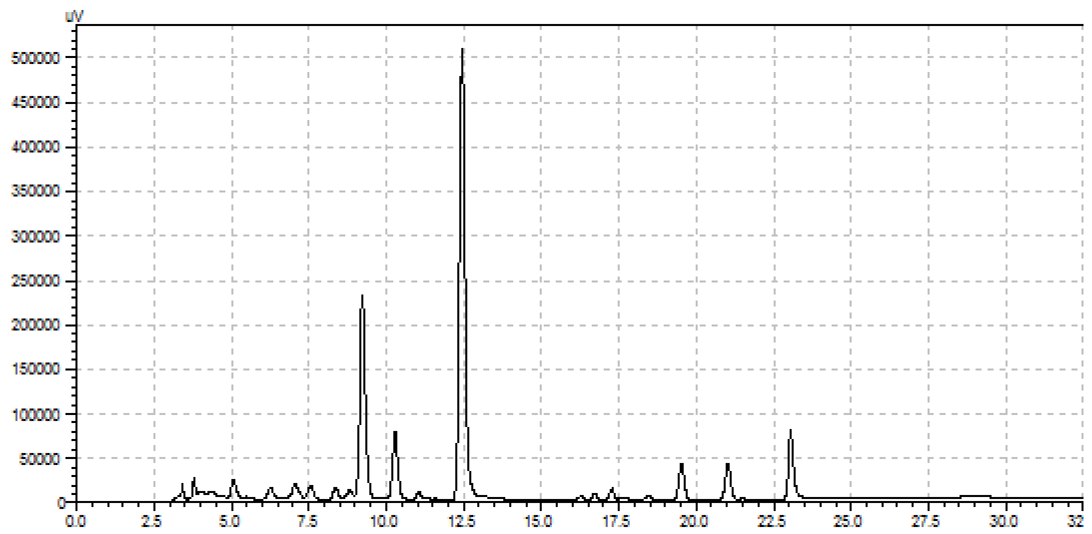

Figura 14 – Cromatograma isoflavonas microencapsulação T3 120°C

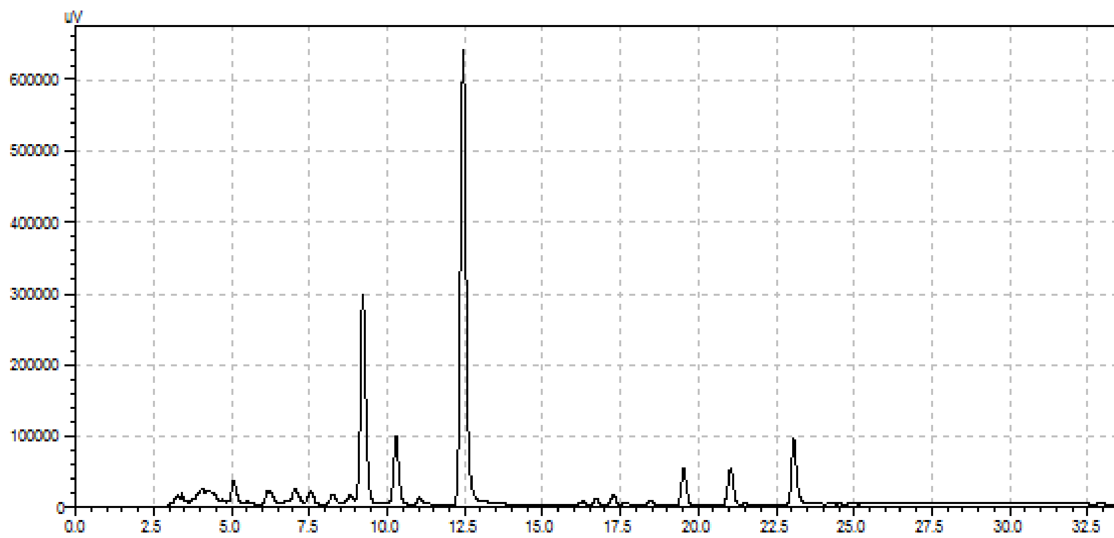

Figura 15 – Cromatograma isoflavonas microencapsulação T3 130°C

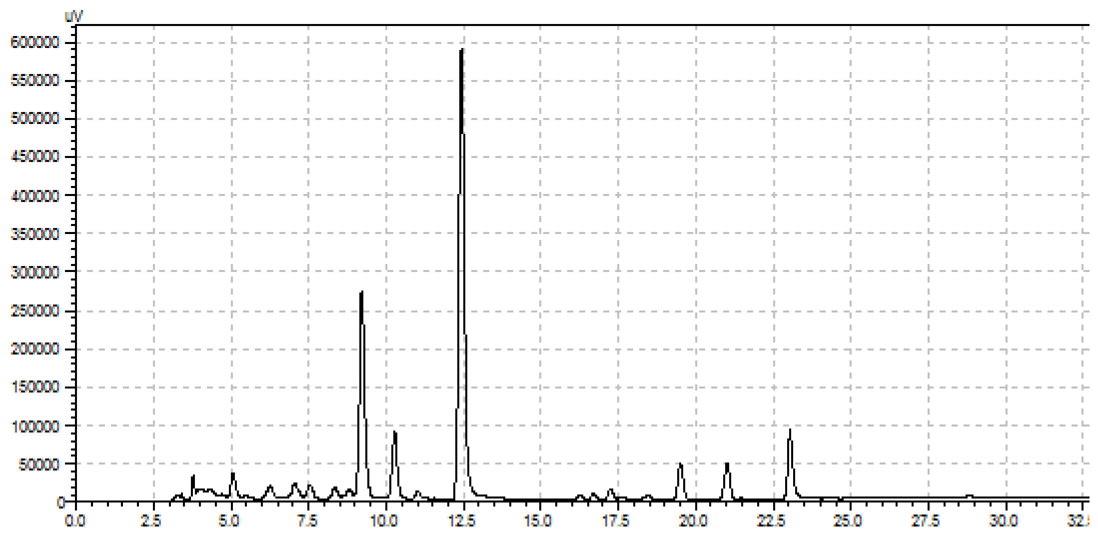

Figura 16 – Cromatograma isoflavonas microencapsulação T3 140°C

Anexo 4 – Termo de consentimento livre e esclarecido

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Você está sendo convidado a participar de um estudo intitulado "Extração, caracterização, microencapsulação das isoflavonas do melaço de soja e aplicação em massa fresca"**.**

Procedimentos a serem realizados

Serão oferecidas a você amostras de massa alimentícia fresca e solicitado que você assinale utilizando a escala hedônica os atributos como: aparência global, cor, odor, sabor e textura. Após você responde a questão se compraria ou não o produto em questão.

Riscos possíveis e benéficos esperados

Fica claro que você não é obrigado a participar do projeto. No caso de recusa você não terá nenhum tipo de prejuízo. A qualquer momento da pesquisa você é livre para retirar-se da mesma. No caso de aceite, fica claro que os produtos oferecidos são seguros e de boa qualidade, não havendo prejuízos ou riscos à saúde, mas poderão ocorrer alguns desconfortos em relação ao sabor e odor na hora de provar. Não haverá benefício financeiro pela sua participação e nenhum custo para você. Você não terá benefícios diretos, entretanto, ajudará a comunidade científica na construção do conhecimento sobre as características sensoriais de um novo produto com maior prazo de validade.

Confidencialidade

Os dados obtidos com esta pesquisa serão publicados em revistas científicas reconhecidas. Os seus dados serão analisados em conjunto com os demais participantes, assim não aparecerão informações que possam lhe identificar, sendo mantido o sigilo de sua identidade. Após a pesquisa os dados serão destruídos.

Utilização dos dados obtidos

Os pesquisadores responsáveis pelo estudo são o Prof. Dr. Cláudia Severo da Rosa e Vanessa Sabrina Fagundes Batista, aluna do Programa de Pós-Graduação e Ciência e Tecnologias dos Alimentos da UFSM. Em qualquer etapa do estudo você terá acesso aos pesquisadores responsáveis pelo estudo para esclarecimento de eventuais dúvidas. Este estudo obteve aprovação junto ao Comitê de ética em Pesquisa da Universidade Federal de Santa Maria, com o protocolo nº (CAAE: 23081.01451/2017-44).

Telefones para contato com a pesquisadora

- Vanessa Sabrina Fagundes Batista (55)99903-2905

Acredito ter sido suficientemente informado a respeito das informações que li ou que foram lidas para mim. Ficaram claros para mim quais são os objetivos do estudo, os procedimentos a serem realizados, seus desconfortos e riscos, as garantias de confidencialidade e de esclarecimentos permanentes. Ficou claro também que minha participação é isenta de despesas. Concordo voluntariamente em participar deste estudo e poderei retirar meu consentimento a qualquer momento, antes ou durante o mesmo, sem penalidades e prejuízos.

Ainda, declaro que obtive de forma apropriada e voluntária o Consentimento Livre e Esclarecido deste sujeito da pesquisa ou representante legal para a participação neste estudo.

______________________________	______________________________
Assinatura do participante	Assinatura do responsável pelo estudo

Santa Maria,________ de__________________________ de 2017.

Comitê de Ética em Pesquisa (CEP/UFSM) – Avenida Roraima, 1000 – Prédio da Reitoria – 7º andar – Sala 702, Cidade Universitária, Bairro Camobi, 97105-900, Santa Maria – RS.

APÊNDICE H – Ficha de avaliação Sensorial

Nome: ____Data: __/__/_
Sexo: ( ) F ( ) M
Idade: ( ) 17 – 30 ( ) 31 – 50 ( ) +51

Você está recendo uma amostra de massa alimentícia fresca, por favor, prove-a e assinale, através da escala, o quanto gostou ou desgostou dos seguintes atributos do produto:
Amostra: ___________

ESCALA	ATRIBUTOS				
	COR	ODOR	SABOR	TEXTURA	APARÊNCIA
GOSTEI MUITÍSSIMO					
GOSTEI MUITO					
GOSTEI					
INDIFERENTE					
DESGOSTEI					
DESGOSTEI MUITO					
DESGOSTEI MUITÍSSIMO					

Em relação a sua intenção de compra você

( ) Certamente compraria
( ) Provavelmente compraria
( ) Tenho dúvidas se compraria
( ) Provavelmente não compraria
( ) Certamente não compraria

Observações:____
____

Obrigada !