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EXTRAÇÃO HIDROETANÓLICA DE ANTIOXIDANTES DE FOLHAS DE *MORINGA OLEÍFERA* LAM E SUA APLICAÇÃO EM SUBSTITUIÇÃO À CONSERVANTE QUÍMICO EM EMBUTIDO CÁRNEO TIPO MORTADELA

MARIA FERNANDA FRANCELIN

Maringá

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"EXTRAÇÃO HIDROETANÓLICA DE ANTIOXIDANTES DE FOLHAS DE *MORINGA OLEIFERA* LAM E SUA APLICAÇÃO EM SUBSTITUIÇÃO À CONSERVANTE QUÍMICO EM EMBUTIDO CÁRNEO TIPO MORTADELA"

Dissertação apresentada à Universidade Estadual de Maringá, como parte das exigências do Programa de Pós-graduação em Ciência de Alimentos, para obtenção do grau de Mestre em Ciência de Alimentos.

Prof. Dr. Benicio Alves de Abreu Filho

Profa. Dra. Raquel Guttierres Gomes

Profa. Dra. Angélica Marquetotti Salcedo Vieira
(Orientadora)

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BIOGRAFIA

Maria Fernanda Francelin nasceu em 18 de outubro de 1983 na cidade de Rondon-Pr. Possui graduação em Engenharia de Alimentos pela Universidade Estadual de Maringá. Tem experiência nas áreas de extração de antioxidantes, atuando principalmente nos seguintes temas: avaliação de processo de extração e aplicação de extratos antioxidantes.

Dedico

A Deus, minha família, noivo, amigos
e orientadoras pelo apoio,
força, incentivo,
companheirismo e amizade.
Sem eles nada disso seria possível.
A vocês meu amor eterno e gratidão.

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APRESENTAÇÃO

Esta dissertação de mestrado está apresentada na forma de um artigo científico

EXTRAÇÃO HIDROETANÓLICA DE ANTIOXIDANTES DE FOLHAS DE *MORINGA OLEÍFERA* LAM E SUA APLICAÇÃO EM EMBURIDO CÁRNEO TIPO MORTADELA.

Autores:

Maria Fernanda Francelin[1], Idinéa Fernandes dos Santos[1], Jesuí Vergílio. Visentainer[2], Andressa Carla Feihrmann[4], Rosangela Bergamasco[3], Thiago Claus[2], Angélica Marquetotti Salcedo Vieira[1]

Instituição:

1Universidade Estadual de Maringá, Programa de Pós-Graduação em Ciência de Alimentos.

2Universidade Estadual de Maringá, Departamento de Química.

3Universidade Estadual de Maringá, Departamento de Engenharia Química.

4Universidade Estadual de Maringá. Departamento de Engenharia de Alimentos.

GENERAL ABSTRACT

INTRODUCTION: In meat products and their derivatives, the processes of lipid peroxidation caused by free radicals caused by oxidation in fats lid to chemical deterioration. The accumulation of compounds provided of this process can result in the development of rancid flavors and color changes. Antioxidant compounds are molecules that prevent the uncontrolled formation of these free radical species and therefore have the ability to retard lipid oxidation process thereby increasing the durability of products. The industries generally use is the addition of synthetic antioxidants to ensure greater durability in their products, but the use of synthetic antioxidants bring harm for the health of consumers, as an alternative to avoid these losses caused by addition of these chemical preservatives is use natural compounds possessing antioxidant, examples thereof are phenols and generally flavonoids. Extracts from leaves of *Moringa oleifera* Lam were reported to exhibit antioxidant activity both in vitro and in vivo, due to the abundance of flavonoids and phenolic compounds, which makes a plant with a high value for this purpose.

AIMS: This study aimed to investigate the effectiveness of antioxidant compounds in the leaves of Moringa oleifera Lam with hydroethanolic solvent extract application in embedded meat, like bologna, with further evaluation of the final product in relation to its effectiveness as a natural antioxidant.

MATERIAL AND METHODS Using the program Design Expert 7.1. delineated and optimized up a sheet of antioxidants extraction process of *Moringa oleifera* Lam which were collected in the experimental farm of the State University of Maringa, dehydrated at a controlled temperature of 30 ° C and exposed to an extraction at room temperature with stirring continuous in periods of time ranging from 0-24 hours with extraction solutions ranging their concentrations from 0-100% ethanol in its composition. The extracts were evaluated for the amount of total phenolic compounds using the methodology described by Singleton and Rossi (1965) and EC50 by DPPH radical scavenging capacity, using the Brand-Williams *et al*. (1995) methodology. The extract with more antioxidant power was determined by Desing Expert 7.1 program through the optimization surface. The extract containing the best antioxidant potential was used in bologna formulations to replace the use of chemical preservative Sodium erythorbate. Specifically, the source of preservative used was 0.25% and 0.00% sodium erythorbate extract (CONT); 0.25% extract and 0.00% sodium erythorbate (M0,25), 0.50% and 0.00% extract erythorbate (M0,50) and 1.00% extract and 0.00 % erythorbate (M1,00). The other ingredients used in the manufacture of bologna was added as a proportion of the total amount of the mixture in the same amount in all treatments. The pellet was embedded in bologna casings of 35 mm caliber plastic polyamide in 100g portions. The bologna were then, baked in an oven with continuous flow of air at a temperature of sequence/time 55 °C/30 min, 65 °C/30 min, 75 °C/30 min, and 85 °C/30 min, such that the center of each portion reached 73 ° C. The bologna were evaluated in relation of lipid oxidation by the TBARS method according to Ohkawa et al (1979), color using a Minolta colorimeter held in colorimeter CR-300 (Konica Minolta, Japan) previously calibrated in a

white surface in accordance to predetermined standards and pHaccording to the methodology AOAC (2003) for 0, 15, 30, 45, 60, 75, 90, 105 and 120 days after production. Regarding the microbiological testing using spread plate methodology tests were done at 0, 30, 60 and 90 days. The proximate analysis using methods described by AOAC (2003) the texture was carried in a texturometer Stable Micro System, TA.XT/Plus/50, Godalming, UK) with specific parameters and probes, according to express itself in the manual method equipment fatty acids according to the method of Hartman and Lake (1973), sensory analysis according to the methodology described by HOUGH, G., *et al*, 2001 with acceptance to the product in relation to the color, flavor, odor and texture, were held on the seventh day. The Oxitest by oxidation analysis (Velp Scientifica, Usmate, Milan, Italy), equipped with two separate oxidation chambers with operating conditions described in the equipment manufacturer's manual and water activity by a water activity meter type AW-500 -TH Sprint, Novasina, according to the procedure described in its manual (Novasina, 2004) were performed on Day 0.

Data analysis was performed using ANOVA and Tukey test, focusing on significant differences among them. Correlations between datas were calculated by SISVAR software statistical program, which was employed with significance between the differences of 5% ($p < 0.05$).

RESULTS AND DISCUSSION: To the extract was found optmized a quantity of phenolic compounds 1333.84 mgGAE / L and the lowest concentration capable of reducing by 50% the amount of DPPH radicals is 12.43 mgGAE / L. These values were found by Desing Expert program 7.1. and validated through Phenolic compounds analysis and DPPH radical sequestration through the methods previously mentioned. For analyzes in the formulations of bologna there were no significant differences in the proximate analysis, moisture, water and color of activity showing that the addition of the extract was not influential in these parameter settings. For pH assay, TBARs and microbiological assay, the extract was efficient in maintaining their values little changed from the passage of time, and the formulations showed no significant difference ($p < 0.05$) compared to quantities added to the different formulations. For the oxidation analysis by Oxitest all samples differed among formulations and the sample had a lower concentration of extracts was shown to be more efficient (M0,25). The addition of the extracts in different proportions in the formulations showed significant interference in the texture of mortadella, and the larger the addition of extract, low texture was presented. This fact can be explained by the removal of sodium erythorbate. For sensory analyze the aroma and flavor parameters the addition of the statement could not be perceived by the judges who did not differentiate the notes given the different formulations, color parameters for all samples were considered different being M1,00 formulation obtained higher note, in the category texture samples also had different notes where M0,25 sample had higher average and overall appearance just M0,50 formulation different from the other.

CONCLUSIONS: The present study demonstrated that the process of extraction of antioxidants from leaves of *Moringa oleifera* Lam using the extracting solution hydroethanol in room temperature is a good source of phenolic compounds, and these compounds scavenging activity present significant free radical and thus a power the presence of these reducing and therefore have the ability to stabilize products of oxidative action. The application of this extract in meat sausages, bologna type, replacing chemical preservatives

was efficient. Furthermore, the addition of the extracts showed no significant effect on some of the reviews in the control parameters. The microbiological quality of samples formulations of mortadella were kept in the course of the analysis of shelf life. These results indicate that the extract of leaves of *Moringa oleifera* Lam can be used as a natural antioxidant to prevent oxidation of lipids in meat sausages.

Keywords: *Moringa oleifera* Lam; Mortadella; Antioxidant Extraction

RESUMO GERAL

INTRODUÇÃO: Em carnes e produtos de seus derivados, os processos de peroxidação lipídica, causada pela oxidação originada por radicais livres em gorduras, causam a deterioração química. O acúmulo dos compostos provenientes deste processo pode resultar no desenvolvimento de sabores rançosos e mudanças na cor. Compostos antioxidantes são moléculas que impedem a formação descontrolada destas espécies de radicais livres e por consequência possuem a capacidade de retardar os processos de oxidação lipídica aumentando assim a durabilidade de produtos. As indústrias de um modo geral utilizam adição de antioxidantes sintéticos para garantir maior durabilidade em seus produtos, porém a utilização de antioxidantes sintéticos trazem prejuízos a saúde do consumidor, assim uma alternativa de evitar estes prejuízos causados pela adição destes conservantes químicos é a utilização de compostos naturais que possuam capacidade antioxidante, exemplos destes compostos são os fenóis e flavonóides de uma maneira geral. Os extratos de folhas de *Moringa oleífera Lam* foram relatados como exibindo atividade antioxidante tanto *in vitro* como *in vivo,* devido à abundância de compostos fenólicos e flavonóides, isto a torna uma planta com alto valor de exploração para este fim.

OBJETIVOS; Este trabalho teve como objetivo investigar a eficiência de compostos antioxidantes presentes no extrato de folhas de *Moringa oleífera* Lam com solvente hidroetanólico em aplicação em embutido cárneo, tipo mortadela, com posterior avaliação do produto final em relação a sua eficiência como antioxidante natural.

MATERIAL E METODOS: Com a utilização do programa Desing Expert 7.1. foi delineado e otimizado um processo de extração de antioxidantes de folhas de *Moringa Oleífera* Lam, colhidas na Fazenda experimental da Universidade Estadual de Maringá, desidratas a temperatura de 30°C e expostas a extração a temperatura ambiente, sob agitação contínua em períodos de tempos que variaram de 0-24 horas com soluções extratoras que variaram suas concentrações de 0-100% etanol em sua composição. Os extratos foram avaliados quanto a quantidade de compostos fenólicos totais utilizando a metodologia descrita por Singleton e Rossi (1965) e Ec50 pela capacidade de sequestro de radical DPPH, utilizando a metodologia de Brand-Williams et. al. (1995). O extrato com maior poder antioxidante foi determinado pelo programa Desing Expert 7.1. através da superfície de otimização.
O extrato contendo o melhor potencial antioxidante foi utilizado em formulações de mortadela em substituição a utilização do conservante químico Eritorbato de Sódio. Especificamente, a fonte de conservante utilizada foi 0,25% de ertirobato de Sódio e 0,00% de extrato (CONT); 0,25% de extrato e 0,00% de eritorbato de sódio (M0,25), 0,50% de extrato e 0,00% de eritorbato (M0,50) e 1,00% de extrato e 0,00% de eritorbato (M1,00). Os demais ingredientes utilizados no processamento da mortadela foram adicionados na mesma proporção da quantidade total da mistura, na mesma quantidade em todos os tratamentos. Com a massa foram embutidos mortadelas em tripas de 35 milímetros de calibre de material plástico de poliamida em porções de 100g. As mortadelas foram então cozidas em estufa com fluxo contínuo de ar em uma sequência de temperatura/tempo de 55 °C/30 min, 65 °C/30 min, 75 °C/30 min, e 85 °C/30 min, de tal modo que o centro de cada uma das porções atingiu 73 °C. As mortadelas foram avaliadas em relação a oxidação lipídica por TBARs seguindo a metodologia de Ohkawa et. al. (1979) , cor utilizando um colorímetro

Minolta CR-300 (Konica Minolta, Japão), previamente calibrado em superfície branca de acordo com padrões pré-estabelecidos e pH segundo metodologia AOAC (2003) aos 0, 15, 30, 45, 60, 75, 90, 105 e 120 dias após a produção. Em relação às análises microbiológicas, utilizando metodologia de pour plate os testes foram feitos aos 0, 30, 60 e 90 dias. As análises centesimais utilizando métodos descritos pela AOAC (2003), textura foi realizada em texturômetro Stable Micro System, TA.XT/Plus/50, Godalming, UK) sendo os parâmetros e lâminas específicas, de acordo com a metodologia expressa no manual do próprio equipamento, ácidos graxos de acordo com o método de Hartman e Lago (1973), oxidação por Oxitest e as análises sensoriais conforme metodologia descrita por HOUGH, G. et. al, 2001, demosntrando aceitação ao produto em relação a cor, sabor, odor e textura, foram realizadas ao sétimo dia. A análise de oxidação por Oxitest, (Velp Scientifica, Usmate, Milão, Itália), equipado com duas câmaras de oxidação separadas, com condições de funcionamento descritas no manual do fabricante dos equipamentos e atividade de água por um medidor de atividade de água do tipo AW Sprint –TH-500, Novasina, de acordo com o procedimento descrito em seu manual (NOVASINA, 2004)foram realizadas no dia 0.
A análise dos dados foi realizada com o teste ANOVA e Tukey com foco em diferenças significativas no meio. Correlações entre os dados obtidos foram calculados pelo programa estatístico de software SISVAR, foi empregado com nível de significância entre as diferenças médias de 5% (p <0,05).

RESULTADOS E DISCUSSÃO Para o extrato otmizado foi encontrado uma quantidade de compostos fenólicos de 1333,84 mgGAE/L e a menor concentração capaz de reduzir a 50% a quantidade de radicais DPPH foi de 12,43 mgGAE/L. Estes valores foram encontrados pelo programa Desing Expert 7.1. e validados através de análises de Compostos fenólicos e Sequestro de radical DPPH através das metodologias anteriormente citadas. Para as análises realizadas nas formulações das mortadelas não houveram diferenças significativas para as análises centesimais, umidade, atividade de água e cor mostrando que a adição do extrato não influenciou nestes parâmetros. Para as análies de pH, TBARs e análies microbiológicas, o extrato se mostrou eficiente em manter seus valores pouco alterados em relação ao decorrer do tempo, e as formulações não apresentaram diferença significativa ($p<0,05$) em relação a concentração adicionada nas diferentes formulções. Para a análise de oxidação pelo Oxitest todas as amostras se diferenciaram entre as formulações e a amostra que possuía menor concentração de extratos, demonstrou-se mais eficiente (M0,25). A adição dos extratos nas diferentes proporções nas formulações mostrou interferência na textura das mortadelas, sendo, quanto maior a adição de extrato, menor textura. Este fato pode ser explicado pela retirada do eritorbato de sódio. Para a analise sensorial os parâmetros aroma e sabor a adição do extrato não foi capaz de ser percebida pelos provadores que não diferenciaram as notas dadas as diferentes formulações, para o parâmetro cor todas as amostras foram consideradas diferentes sendo a formulação M1,00 que obteve maior nota. No quesito Textura as amostras também obtiveram notas diferentes onde a amostra M0,25 obteve maior média e para aparência global apenas a formulação M0,50 se diferenciou das demais.

CONCLUSÕES. Este resultado mostrou que este método de extração de antioxidantes de folhas de *Moringa Oleífera Lam*, utilizando as condições escolhidas no presente estudo, resultou em um extrato contento os compostos de interesse contendo capacidade antioxidante ativa. Deste modo, concluiu-se que a planta em estudo é uma boa fonte de compostos fenólicos, sendo estes compostos com atividade de eliminação significativa de radicais livres presentes no meio de aplicação. A aplicação deste extrato em embutidos cárneos, tipo mortadela, em substituição a conservantes químicos mostrou-se eficiente. Além disso, a adição dos extratos não apresentou efeito significativo sobre os parâmetros de cor, textura e avaliação sensorial em relação ao controle. A qualidade microbiológica das amostras de mortadelas foram mantidas no decorrer das análises de shelf life. Estes resultados indicam que o extrato de folhas de *Moringa Oleífera* Lam pode ser usado como um antioxidante natural, para impedir a oxidação de lípidos em embutidos de carne.

Palavras chaves: *Moringa Oleífera* Lam, Mortadela, Antioxidante, Extração

HYDRO-ETHANOL EXTRACTION OF ANTIOXIDANTS OF *Moringa oleífera* Lam. LEAVES AND ITS APPLICATION IN MORTADELLA-TYPE MEAT PRODUCTS

Maria F. Francelin[1], Idinea F. dos Santos[1], Jesuí V. Visentainer[2], Andresa C. Feihrmann[4], Rosangela Bergamasco[3], Thiago Claus[2], Angélica M. S. Vieira[1]*

1. State University of Maringa, Postgraduate Program in Food Science

2. State University of Maringa, Department of Chemistry.

3. State University of Maringa, Department of Chemical Engineering

4. State University of Maringa. Department of Food Engineering.

Av. Colombo, 5790 - Jardim Universitário, Maringá - PR, 87020-900

Phone: 55 44 30115093 - Fax: 55 44 30114531

*E-mail: amsvieira@uem.br

ABSTRACT – Hydro-ethanol extracts of the leaves of *Moringa oleifera* were evaluated for antioxidant capacity so that their most efficient use as a natural preservative could be detected. The extracts of *Moringa oleifera* leaves were evaluated by quantification methods of total phenolic compounds and evaluation of EC50 by the scavenging capacity of DPPH radical. Analyses and results were outlined and optimized by the statistical program Design Expert 7.1. The extract with the highest antioxidant activity rate (EC50 = 12.43%) was applied to chicken mortadella formulations with 0.25%, 0.50% and 1.00% extract concentrations; control consisted of a sample with 0.25% sodium erythorbate. The different mortadella formulations were evaluated by centesimal, microbiological, sensory and lipid oxidation analyses for 120 days. There was a lower lipid oxidation rate in the formulation with 0.25% extract. Results showed that the extract could inhibit lipid oxidation.

Keywords: *Moringa Oleifera;* antioxidant capacity; mortadella.

1. INTRODUCTION

In meat and its derivatives, lipid peroxidation processes, caused by oxidation originated by free radicals in fat, cause chemical deterioration due to the production of Reactive Oxygen Species (ROS), such as peroxyl, superoxide, singlet oxygen and hydroxyl radicals[1]. The accumulation of these compounds from the process, such as alcohols, aldehydes, ketones, esters and others, which are generally volatile, results in the development of rancid flavors and color changes that may also affect the solubility of the meat´s protein and reduced nutritional value[2].

Antioxidant compounds are molecules that prevent the uncontrolled formation of these free radical species and therefore possess the ability to slow down lipid oxidation processes therefore increasing durability of the products in which they are applied[3] .

According Nkukwana *et al*.,[4] three main factors define the susceptibility of lipids to peroxidation in the tissue, namely, the proportion of PUFA in lipid bilayers, the amount of reactive oxygen species produced and the level of endogenous or nutritional antioxidants. The authors enhance that practically the addition of antioxidants, which are organic molecules of synthetic or natural origin capable of scavenging the active forms of oxygen involved in the initiation stage or progression of oxidation, is the major preventive measure against lipid oxidation in meat and meat products.

In fact, industries generally employ the addition of synthetic antioxidants to ensure the products´ greater durability. Since the commercially available synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), jeopardize consumers´ health, their use has to be limited to restricted amounts due to their toxicity to vital organs[1]. One way to avoid these losses may be the use of natural plant-derived antioxidants. In fact, they have received considerable attention due to their ability to preserve food and prevent oxidation-caused rancidity. Examples of such compounds include phenols, flavonoids, proanthocyanidins, flavonoids, vitamin C, vitamin E and β-carotene, with high antioxidant potential[2-5-6]. This trend is reflected worldwide and recently the European Union issued Directive 2006/52 / EC to reduce nitrites in meat sausages[7].

The above issues indicate the need for research on the use of natural additives as alternative methods to extend the shelf life of products. Leaf extracts of *Moringa oleifera* Lam (commonly called Moringa) were reported to exhibit antioxidant activity both *in vitro* and *in vivo* due to their many phenolic compounds and flavonoids[8-9]. Some researchers confirmed that the leaves of Moringa have high levels of chlorogenic acid, gallic acid,

kaempferol and quercetin glycosides[10-11], or rather, it is a high-rated plant for precisely this end. The Moringa is a fast-growing shrub of the family Moringaceae, native to India, Pakistan, Bangladesh and Afghanistan, and widely used in folk medicine in these regions[12]. The benefits and high nutritional value of the Moringa leaves, fruits, flowers and roots are currently well-known, whilst their use and preparations are highly heterogeneous and may be consumed as food[13], as medicine[12] and as nutritional supplements[14]. Several types of phytochemicals, carotenoids, alkaloids, flavonoids, and phenolic compounds may be found in all parts of the plant[15]. These compounds have important properties since they contain antioxidant characteristics which may be widely used by pharmaceutical to food industries[9-16-17]. Awodele *et al*.[18] studied aqueous extracts from the Moringa leaves in mice food and concluded that the extract is not in any way lethal when administered orally at various doses ranging between 400 mg / kg and 6.4 g / kg.

Current study investigates the most efficient extraction method of the antioxidant compounds with hydro-ethanol solvent in 24 hours of extraction, at the most, and apply the extract with the best antioxidant potential in meat products, such as mortadella, coupled to the evaluation of the final product with regard to its efficiency as a natural antioxidant.

2. MATERIALS AND METHODS

2.1. Collection and drying of *Moringa oleifera* leaves

Moringa leaves were harvested on the Experimental Farm of the State University of Maringa, Maringá PR Brazil, in winter, and within a 7-day rainless period. The leaves were washed under running water; water excess was drained out; the leaves were dehydrated in a dehydrating device with continuous flow of air at a controlled temperature of 30°C. Mass measurements were taken every hour, until they achieved constant weight.

The moisture of leaves was calculated by Equation 1:

$$U(\%) = \frac{(Pi-Pf)}{Pi} x100 \qquad (1)$$

where:

Pi = initial mass

Pf = final mass

The dried leaves were ground in a home processor and the powder was stored in a pot with an airtight lid and stored at room temperature, in the dark.

2.2. Antioxidant extract of the *Moringa oleifera* leaves

Further, 0.75g of leaves were weighed for 15 ml of extracting solution at a ratio of 1:20 w/v. The mixtures were continuously stirred during the whole period of extraction, at room temperature (25±2 °C), at periods and concentrations of extraction, following Table 1. Water and ethanol were the extracting agents. The solutions were then centrifuged at room temperature (25±2 °C), for 15 minutes, at 6000 rpm. The supernatant extracts were subjected to physicochemical analysis immediately after this procedure. The extract used for the manufacture of the mortadella was obtained at the time of formulation.

2.3. Experimental design for the best antioxidant extraction process of *Moringa oleifera* leaves

The binomial response surface method is a mathematical and statistical tool that has been widely used for optimizing various parameters of a production process[19].

A method with five variables of time and concentration of solution extractors, independent of one another, was used to optimize the extraction process. The independent variables comprise concentration of 0-100% ethanol (v/v) and extraction time 3-24 hours, determined by computer program Design Expert (version 7.1).

The rates of total phenolic compounds and antioxidant activity were selected as the response of experiments. Data were analyzed by the program and the coefficients were assessed by F-value to optimize the experimental conditions of the rates of total phenolic

compounds and antioxidant activity. Regression analysis ($p < 0.05$) and response surface were performed by the analysis of variance (ANOVA). Data are shown in Table 2.

2.4. Determination of total phenolic compounds of extracts of *Moringa oleifera* leaves

The amount of total phenolic compounds in the samples of Moringa leaf extracts was determined by colorimetric analysis with Folin-Ciocalteu reagent, described by Singleton & Rossi[20]. Moreover, 8.4 ml of extraction solution of the sample, 100 µL of extract and 500 µL of reactive Folin-Ciocalteu reagent were added in a test tube. After 3 min, 1.0 mL of saturated sodium carbonate was added in each tube and the tube was vortex-stirred for 10 sec. After 1 h, the absorbance was measured with a spectrophotometer at a 725 nm wavelength. Measurement was compared with an acid calibration curve and the results were expressed in mg of gallic acid equivalents (GAE) per liter (mg GAE / L).

2.5. Determining the sequestration capacity of DPPH radical and EC50 calculation.

The scavenging capacity of DPPH was determined according to Brand-Williams *et al*.[21], with modifications. Ten concentrations of each extract for each sample were tested to obtain their curves. Since the method works equally well with methanol or ethanol[22], ethanol was chosen because of its low toxicity and because it is the same compound used for the extractions. Further, 200µl of sample or extraction solution (blank) were added to 3.3 ml of a 50 µM DPPH in ethanol, prepared daily. The amount of initial DPPH was chosen for the initial and final absorbance spectrophotometry within the accuracy range[23] and the volume of the selected sample is adequate to obtain results within the required range for calculating 50% of the sequestration of the radical.

The absorbance was read at 515 nm by a spectrophotometer after 30 minutes. The reaction was carried out at room temperature (25±2°C). For each concentration of test sample, the radical scavenging capacity was obtained by Equation 2.

Scavenging concentrations of 50% of DPPH radical and EC50 values of test compounds were calculated.

$$\% \text{ Inhibition of DPPH} = [1- (Aa \div Ab)] \times 100 \quad (2)$$

where:

Aa = absorbance of the sample

Ab = absorbance of DPPH solution

2.6. Preparation of mortadella

Chicken mortadella formulations were prepared with 0.00% (CONT), 0.25% (M0.25), 0.50% (M0.50) and 1.00% (M1.00) antioxidant extract of Moringa leaves in their formulations; a control diet was prepared without any extract. Table 1 provides the other ingredients used in the manufacture of mortadella. The mortadella were processed in Block O 27, Department of Food Engineering, located at the State University of Maringa - Maringa PR.

The ingredients used in the manufacture of mortadella were added at a proportion of the total amount of the mixture in the same amount in all treatments, as follows: Mechanically deboned chicken meat GTFoods Group; boneless and skinless chicken breast meat (Copacol, Brazil); cassava starch and textured soy protein (Yoki); curing salt and flavoring for ready-for-consumption commercial mortadella (0.4 %) (Ibrac, Brazil); sodium erythorbate, phosphate sodium, garlic powder and white pepper powder (Kienast & Krastchemer Ltda); sodium chloride (Cisne, Brazil); monosodium glutamate (Ajinomoto, Brazil).

The ingredients were added to the emulsifying cutter (Frigomaq-Frigo Industrial Ltda, Chapecó SC Brazil) in the following order: a) chicken breast meat and salts; b) half the water/ice and MSM; c) the other half of ice/water; d) cassava starch and textured soy protein.

The emulsification of the ingredients was carried out at temperatures below 13°C until a homogeneous paste consistency was obtained.

The mortadella was processed in 35 mm plastic polyamide packaging containing 100 g each. The mortadella were then cooked in a buffer with a continuous air flow, at a temperature of sequence/time 55°C/30 min, 65 °C/30 min, 75°C/30 min and 85°C/30 min, such that the center of each portion reached 73 °C. At the end of the cooking process, the mortadellas were cooled in water at 5°C, for 30 min. The products were packed in polyethylene bags, packaged and kept at 4°C. Each formulation was prepared in triplicate (three batches with a total of 3 kg per treatment).

The mortadellas were evaluated for TBARS lipid oxidation, color and pH at 0, 15, 30, 45, 60, 75, 90, 105 and 120 days after production. Microbiological tests were conducted at 0, 30, 60 and 90 days. The centesimal, texture, oxidation and sensory analyses were performed on the seventh day. The oxidation analysis Oxitest and water activity were carried out on day 0.

2.7. Centesimal composition

The analyses of moisture, fat, protein and ash rates of mortadella samples were performed in triplicate for each lot, following AOAC[24]. Amount of protein was calculated by the micro-Kjeldahl method with a digestion block and a nitrogen distiller. Fat was determined according to method by Bligh and Dyer[25]. Moisture rate was determined in a buffer at 105°C until a constant sample weight. Mineral residues (ashes) were determined by carbonization and incineration of the samples in a muffle furnace at 550°C.

2.8 The water activity (Aw), pH, TBARS and Oxitest

Water activity (Aw) was determined at room temperature by a water activity meter sprint - TH type AW-500, Novasina, according to instructions by manufacturer. Analysis was done in triplicate for each formulation, only for zero time samples.

Moreover, pH rates were measured (Quimis Q-400 pH meter) with a universal electrode, according to AOAC[24] method, and analyses were performed on all samples fortnightly for 120 days.

The analyses of Thiobarbituric acid reactive substances (TBARS) of the products were made in triplicate fortnightly for 120 days to evaluate lipid oxidation, following method by Ohkawa *et al*.[26]. TBARS were calculated in mg malondialdehyde per kg / sample.

Forced oxidation assays of mortadella formulations were conducted according to Verardo *et al*.[27] with a reactor called "Oxitest" (Velp Scientifica, Usmate, Milan, Italy) equipped with two separate oxidation chambers. The specific sample was placed in a chamber. The apparatus was sealed and heated to a certain temperature and oxygen was injected into the chamber until a predetermined pressure of oxygen was reached. When oxygen was added, the chamber was electronically locked and testing started. Since any oxidable compound reacts with oxygen in the chamber, the gas pressure inside was reduced. The chamber pressure was monitored throughout the process and the induction period (IP) was obtained using the two-tangent method. The Oxitest method is therefore effective to assess the capacity of a given compound to retard or inhibit the oxidation of a given substrate. All tests were performed at 90°C, with an initial oxygen pressure of 6 bars and 99.99 % purity. Samples of 5 g of mortadella formulations were evaluated.

2.9 Fatty Acid Analysis

For the analysis of fatty acids, approximately 100 mg of lipid were transmethylated according to method by Hartman and Lago (1973)[28], with ammonia solution and sulfuric acid in methanol as esterifying agent. Esters of fatty acids were isolated and analyzed by gas

chromatography Agilent 7890A Model, coupled to a mass detector Agilent 5975C, using a column ZB-Glycol Polyethylene Wax (30 m long x 0.25 mm internal diameter x 0.25 mm thick film). The carrier gas was helium (He) and the injection flow rate was 1 mL / min split 1:10. The initial column temperature was set at 50°C, held for 2 minutes, then increased to 220°C at a rate of 4°C/min and held for 7 minutes. The injector temperature was 250 °C. The identification of fatty acids was performed by comparing retention times of the methyl esters of the samples with those of authentic standards (Sigma) at zero time production and in all formulations.

2.10 Color evaluation

Color quantifying was performed by Minolta colorimeter CR-300 (Konica Minolta, Japan) previously calibrated on a white surface, following pre-established standards.

Rate was determined by observations of the exposed internal surfaces to the different mortadella formulations, evaluating the difference between formulations and time.

Three color parameters were evaluated: L*, a* and b*. The rate of a* characterizes the region from red (+a*) to green (-a*); the rate of b* indicates the color range between yellow (+b*) and blue (-b*). The rate L provides brightness, ranging from white (L=100) to black (L=0).

The analyses were performed monthly for 120 days to evaluate the degradation of color through time and by comparing the formulations.

2.11 Cutoff point analysis

Texture measurement was performed by a cutoff point analyzer Stable Micro System, TA.XT/Plus/50, Godalming, UK, with specific parameters and blade following methodology and instructions by manufacturer. The inner nuclei of the mortadella samples for each treatment were assessed. The mortadellas were cut (12 mm diameter and 10 mm height) in

triplicate and compressed to 50 % of their original height, at room temperature, with an aluminum foil compression tube.

The results were expressed in Newton for maximum force required to cut the sample; the analyses were performed at time zero preparation for all formulations.

2.12 Sensory analysis

The samples were subjected to a sensory panel composed of 120 untrained male and female judges, aged between 18 and 54 years, selected among students, teachers and staff of the State University of Maringa, Maringá PR Brazil. The tasters/judges, asked to evaluate the sensory attributes of the product and purchase intent, received 10 g of mortadella from each treatment, with a three-number code, coupled to 50 mL of water in disposable plastic cups. The sensory evaluation was performed in individual rooms with white light. Judges were instructed to cleanse their mouth with water between sample-taking. The taster evaluated samples from the 4 treatments, filled the evaluation form by using a nine-point hedonic scale, ranging from 1 (I dislike it very much) to 9 (I like it very much) to evaluate the flavor, firmness and color of each sample[29]. The sensory attributes evaluated for mortadella comprised an overall appearance of the product surface, color, flavor, texture, odor and general acceptability. (CAAE: 27918914.7.0000.0104. N. 681 807)

The products´ Acceptability Index was calculated by Equation (3):

$$AI\ (\%) = A \times 100/B \qquad (3)$$

where:

A = average score obtained for the product;

B = maximum score given to the product.

The AI ≥ 70 % have been used to classify the acceptance of new products undergoing sensory analysis.

2.13 Microbiological analysis

Samples of mortadella formulations were collected, with five replications each. The samples were then placed in sealed bags and packed in insulated boxes and immediately analyzed.

Microbiological analyses determined coliforms at 35 °C and 45°C, *Escherichia coli*, *Staphylococcus* positive coagulase, *Salmonella spp.* and Clostridium sulfite reducer, by spread plate count methodology [30].

The analyses were performed monthly for 120 days.

2.14 Statistical analysis

Data analysis was performed with ANOVA and Tukey´s test, focusing on significant differences in the medium. A completely randomized design was analyzed with a mortadella unit from each lot as an experimental unit for each treatment.

Correlations between data were calculated by SISVAR statistical program with 5 % significance difference ($p < 0.05$). All analyses were performed in triplicate and the results were expressed as averages.

3. Results and Discussion

3.1. Humidity, total phenolic compounds, radical sequestration capacity DPPH and EC50 of the leaves of *Moringa oleifera* Lam

The humidity rate of Moringa leaves was 52.12 %. Nkukwana *et al*.[4] reported 78.3 % and 76.00 % when the natural drying procedure was employed. Rate was lower than that found by Nkuwana *et al*.[4], since the difference in soil composition, climate, local water system of the compared cultivars, and methodology might change the results.

Rates of the phenolic compounds, antioxidant capacity and EC50 were analyzed by the computer program Design Expert 7.1 to calculate the optimal point of extraction for each analysis. Table 2 shows the responses of each variable. Rates were analyzed by computer

program that outlined optimization responses surfaces. Figure 1 provides the graphs. Surfaces revealed that the best outcome for phenolic compounds was the extraction made with 100 % water solvent in 7 h 28 min (448 min), with the expected result of 1846.42 mg GAE/L; in the case of EC50, the best extraction point was with solvent comprising 77% water and 23% ethanol v/v, with extraction time 7 h 55 min (475 min) and expected result 10.51 %. DPPH and EC50 analyses were performed in triplicate according to time and with extractor solutions described above. The phenolic compounds results were 1333.84±0.71 mgGAE/L and EC50 12.43±0.48 mgGAE/L.

Shah *et al*.[31] reported between 46.13 and 49.45 mg GAE/g of phenolic compound extract in the aqueous extracts of Moringa leaves. The authors made the extraction from dried leaves in boiling water. The mixture was kept at room temperature for one hour and filtered; the residue of the leaves was once more extracted and the two extracts were mixed and lyophilized. Das *et al*[32] extracted phenolic compounds from Moringa leaves with water as solvent and processed with Soxhlet for 18-20 h. Total phenolic compounds of the aqueous extract of Moringa leaves were 48.36 mgGAE/g.

Since these polyphenols were responsible for antioxidant activity, data showed that the hydro-ethanol extraction made with the extraction solution was less efficient, possibly due to the fact that phenolic compounds were not exposed to higher temperatures, and extraction probably failed. In the case of EC50 analysis, the extract was also less efficient than the capacity in the aqueous fraction of Moringa leaf extract analyzed.[9] In fact, Verma *et al*.[9] reported 1.193 mg extract/mL, while the extract in current study reached 12.43 mg extract/mL.

The extracts tested in the above-mentioned trials were performed in more extreme conditions, since higher temperatures and serial extractions were employed. These factors may only increase the amount of phenolic compounds in the extracts.

On the other hand, the process in current study used the extraction process at room temperature, highly important when evaluating costs and simplicity of the process. One of the goals of current assay is the application of the extract in a food system and the evaluation of its antioxidant efficiency in the system, or rather, the efficiency of the extract in the system under analysis.

According to the data obtained in the program, the accepted error for total phenolic compounds was 62.64 %; the error reported was 27.76 %; in the case of EC50, the accepted error was 22.29 % and that registered was 12.43 %. The process was thus validated.

Current study demonstrated that the extraction process of antioxidants from leaves of Moringa, using hydro-ethanol extraction solution at room temperature, provided an extract with the required compounds and active antioxidant capacity. Results demonstrate that the plant is a good source of phenolic compounds since the compounds have a significant radical scavenging activity and reducing power.

3.2. Centesimal analysis of mortadella

Table 3 demonstrates the chemical composition of mortadella. Since there was no significant difference ($p<0.05$) between treatments, the extract was incapable of change these parameters.

Moisture, lipid and protein rates in each formulation lie within standards established by Brazilian law. Requirements of Instruction 4 of the Department for the Inspection of Animal-Derived Products (DIPOA)[33] for mortadella are a minimum of 12 % protein and a maximum of 65 % humidity and 30 % fat.

3.3 Variation of the pH, TBARS and Oxitest during the storage period

The pH and TBARS analyses evaluated variations in formulations and with regard to time during the storage period. Results are shown in Table 4.

The pH rates for samples treated with Moringa leaf extracts showed no significant differences ($p<0.05$) when compared to control. There was also no significant difference for the storage period. The above demonstrates that the addition of the extract achieved the same control behavior during the storage period. Al-Abdullah *et al*.[34] reported similar pH rates, namely, 6.3-6.8, in their study on chicken mortadella, corroborating data above.

The TBARS test assesses the potential parameter related to the formation of rancid compounds derived from lipid oxidation. Studies by Greene & Cuzume[35] showed that, in the case of meat, TBARS rates needed to produce a sensory perception related to lipid oxidation lay between 0.6 and 2.0 mg malondialdehyde /kg in cooked meats; with rates ≤ 1.0 mg corresponding to products with imperceptible changes or almost imperceptible in oxidation[36]. Rates ≤ 1.6 mg were low for perception sensory. Since in current analysis, TBARS averages in all treatments and storage time were lower than 0.24 mg, the occurrence of oxidation reactions was lower than that considered perceptible to taste.

Oxitest proved to be a very effective method to compare formulations with and without the addition of the extract. Table 5 shows test data by the Oxitest reactor. The control sample (CONT) starts oxidization at 376 ± 7.0 min; the formulation with 0.25 % extract (M0.25) slows oxidation by 344 min; M0.50 formulation slows oxidation by 78.5 min; M1.00 formulation retards oxidation extract by 53.5±7 min. Tests were done in triplicate for each formulation. Result showed that, although the substitution of sodium erythorbate by Moringa leaf extract provided statistically different results ($p<0.05$), samples decrease their capacity in oxidation retarding when the addition of the extract is high. In other words, an increase in the extract addition causes a reduction in the antioxidant capacity of the final product.

The antioxidant mechanism of natural compounds is still far from being fully understood and requires further investigation: for example, whether phenolic compounds may act as pro-oxidants in the presence of some phenolic ions [37]. Jung *et al*.[38] reported optimal

concentrations of 100 mg/kg for α, 250mg/kg for γ and 500 mg/kg for δ tocopherols to enhance the oxidative stability of purified soybean oil and stored in dark, at 55 °C. In fact, these compounds showed significant pro-oxidant effects at concentrations above these levels, because they are labile in the presence of oxygen, light and heat. It may be a possible explanation for the results in the samples when analyzed for oxidative stability.

The application of this extract in mortadella-like processed meat replacing chemical preservatives was efficient. Further, the addition of extracts showed no significant effect on color parameters, texture and sensory evaluation when compared to control. The microbiological quality of mortadella samples was maintained throughout the analysis of shelf life. Results indicate that Moringa leaf extract may be employed as a natural antioxidant to prevent oxidation of lipids in processed meat.

3.4 Lipid Profile

Table 6 shows rates for fatty acids in all samples of mortadella in all formulations. According to Yinggang *et al*.[39], the rates and composition of total lipids in chicken meat are not only related to chicken strains but also to feed conditions, including food and water, to the cooking process. In fact, these parameters may be modified.

All mortadella prepared in the experiment, the meat used in matrix formulations and alllots, characteristics were maintained regardless of race, breeding and feeding region, which enabled to maintain the initial lipid profile of the product to enable the realinteraction between the extract and the product.

Results showed that the rates in current study are close to those registered by Yinggang *et al*.[39] who compared the levels of fatty acids in different chicken strains. Results demonstrated that extracts, regardless of their concentrations, did not alter the amount of the compounds in the final products assessed ($p<0.05$). An inconvenience due to the high amounts of long chain PUFA is their high susceptibility to oxidation, causing a loss of natural

antioxidants and the production of undesirable compounds (from the toxic and sensory point of view) and a decrease in shelf life[40].

3.5 Assessment of the color of mortadella

The interaction of different color formulations had no significant changes between formulations ($p<0.05$), or rather, the addition of the extract at the concentrations tested was not able to alter these parameters. Change in color parameters during the storage period went unnoticeable for each formulation of mortadella.

The color of meat products is a relevant parameter which affects consumers´ acceptance. It is considered as an indicator of quality for the product´s shelf life[41]. The fact that Moringa leaves extracts did not change the color of the final products is very important. Table 7 provides these rates.

3.6 Cutoff Point Analysis (TA) and Aw

The addition of the extracts at different proportions in the formulations showed significant interference on the texture of mortadella. The higher the addition of the extract, the less cutoff point was presented. This fact may be due to the replacement of sodium erythorbate, which, besides being an antioxidant agent, also improves the texture of the final product.

Rates for cutoff point of samples are given in Table 8.

Herrero *et al.*[42] evaluated different formulations of mortadella mainly constituted of pork, beef and chicken and turkey meat. Texture was 4.86±1.25 in mortadella with prevalent pork formulations.

In current assay, Aw rates did not change by adding extracts to the different formulations.

3.7 Sensory analysis of mortadella

In the course of sensorial analysis, respondents identified differences ($p<0.05$) in flavor, texture, odor, color and overall acceptability among the mortadella formulations (Table 9).

There was no significant difference between the samples with regard to flavor. When flavor and aroma were compared, the tasters could not perceive the changes between the formulations. Averages were above or very close to 7.0, expressing the feeling that the product was satisfactorily liked.

In the case of color, the samples with the Moringa extract proved statistically equal but different from control. Within this divergence, the sample with the highest average acceptance was sample M1.00. Since it contained higher amounts of extract, the above datum revealed that the colored compounds in the extract acted positively on the product´s color. There was no significant difference between the parameters (a*, b* and L) in the color analysis with colorimeter.

In the case of texture, there were differences between the sample M0.50 and the others. This result may also be due to untrained tasters.

In overall appearance, only the M0.50 sample proved to be different from the others, resulting from the analysis of conditions or untrained tasters.

There was no negative effect on the addition of extracts. In fact, when the acceptability index to find each attribute was calculated, values higher than 70 % occurred. Actually it was a positive point that the product was accepted[29].

3.8 Microbiological analysis

Table 10 shows rates for microbiological analysis, with results for thermo-tolerant coliforms, total coliforms and clostridium sulfite reducer. All results comply with current Brazilian legislation and with Regulation (EC) N. 2073/2005 of the European Parliament[43] of 15 November 2005 concerning the legislation for marketing products for human consumption

in the European Union, featuring absence of Salmonella and a maximum of $5.0x10^3$ CFU/g product for *E. coli*.

There was no change for *Staphylococcus aureus* analysis (coagulase positive) in samples with different extract additions and no variation in rates over time. In fact, results are within the limits established by Norm EC 2073/2005[43]. The same behavior was observed for the analysis of *Escherichia coli*, with negative results for all samples, over time and in relation to different formulations. *Salmonella spp*. was absent in all analyses.

For the analysis of Thermotolerant coliforms (45 °C) and total coliforms (35 °C), samples containing Moringa extracts showed higher efficiency in controlling of these microorganisms and the results found between the control sample and the statistically different ($p < 0.05$), but not hove a change in the concentration of extract used. The results for these parameters showed no significant difference during the storage time.

Results show that the extract was effective for microbiological control, with rates below the maximum allowed for all concentrations used. Ratshilivhaa[44] evaluated the antimicrobial capacity and fungal extracts from Moringa leaves and showed that most samples harvested during the winter also had fungicide and bactericide effects and could be an antimicrobial remedy for the treatment of infections caused by *E. coli*, based on their low MIC (minimum inhibitory concentration) and full high activity. These results corroborate the data provided in current study.

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4. References

1. Siddhuraju, P.; Becker, K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *Journal of Agriculture and Food Chemistry*, **2003**, 51, 2144–2155.
2. Aqil, F.; Ahmad, I.; Mehmood, Z. Antioxidant and free radical scavenging properties of twelve traditional used Indian medicinal plants**.** *Turkish Journal of Biology*, **2006**, 30, 177–183.
3. Yazdanparast, R.; Ardestani, A. *In vitro* Antioxidant and free radical scavenging activity of *Cyperus rotundus.* *Journal of Medicinal Food*, **2007,** 10, 667–674.
4. Nkukwana, T T; Muchenjr, V; Pietrse, E; Masika, P J; Mabusela, T P; Hoffman, L C; Dzama, K; Fatty acid composition and oxidative stability of breast meat from broiler chickens supplemented with *Moringa oleifera* leaf meal over a period of refrigeration, *Original Research Article, Food Chemistry*, **2014**, 142, 255
5. Cai, Y.; Luo, Q.; Sun, M. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with cancer. *Journal of Life Sciences*, **2004**, 74, 2157–2184.
6. Okwu, D.E. Phytochemicals and vitamin content of indigenous species of South Eastern Nigeria. *Journal of Sustainable Agriculture and Environment*, **2004** 6, 30–34.
7. DIRECTIVE 2006/52/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 5 July **2006,** amending Directive 95/2/EC on food additives other than colours and sweeteners and Directive 94/35/EC on sweeteners for use in foodstuffs.
8. Chumark, P.; Khunawat, P.; Sanvarinda, Y.; Phornchirasilp, S.; Morales, N.P.; Phivthong-ngam, L.; Ratanachamnong, P.; Srisawat, S.; Pongrapeeporn, K. The *in vitro* and *ex vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities

of water extract of *Moringa oleifera* Lam. Leaves. *Journal Ethnopharmacol*, **2008**, 116, 439–446.

9. Verma, A. R.; Vijayakumar, M.; Mathela, C. S.; Rao, C.V.; *In vitro* and *in vivo* antioxidant properties of different fractions of *Moringa oleifera* leaves. *Food Chemistry Toxicol*, **2009**, 47, 2196–2201.
10. Bennett, R. N.; Mellon, F. A; Foidl, N.; Pratt, J. H.; Dupont, M. S.; Perkins, L.; Kroon, P.A. Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *Moringa oleifera* L. (horseradish tree) and *Moringa stenopetala* L., *Journal Agriculture Food Cheistry*, **2003,** 51, 3546–3553.
11. Brahma, N. S.; Singh, B. R.; Singh, R. L.; Prakash, D.; Dhakarey, R.; Upadhyay, G.; Singh, H. B. Oxidative DNA damage protective activity, antioxidant and anti-quorum sensing potentials of *Moringa oleifera. Food Chemistry. Toxicol*, **2009**, 47, 1109–1116.
12. Fahey, J. W. *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties – part 1, *Trees for Life Journal*, **2005**.
13. Lockelt, C. T. C. C; Grivetti, L E. Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought. Study of rural Fulani, Northeastern Nigeria. Int. *Journal Food Science Nutrition*, **2000**, 51, 195-208.
14. Monera and Maponga. *Moringa oleifera* supplementation by patients on antiretroviral therapy. *Journal of the International AIDS Society*, **2010**, 13, 4, P188.
15. Anwar, F.; Rasshid, U., Ashraf, M., Saleem, M., Yusup, S. Application of response surface methodology for optimizing transesterification of *Moringa oleifera* oil: Biodiesel production. *Energy Conversion and Management*, **2011,** 52, 3034–3042.
16. Gasparotto, A. Jr.; Gasparotto, F. M.; Lourenço, E. L. B.; Crestani, S.; Stefanello, M. E. A.; Salvador, M. J.; Silva-Santos, J. E.; Marques, M. C. A.; Kassuya, C. A. L. Antihypertensive effects of isoquercitrin and extracts from *Tropaeolum majus* L.:

evidence for the inhibition of angiotensin converting enzyme. *Journal Ethnopharmaco*, **2011**, 134, 363–372.

17. Soromou, L. W.; Chen, N.; Jiang, L.; Huo, M.; Wei, M.; Chu, X.; Millimouno, F. M.; Feng, H.; Sidime, Y.; Deng, X. Astragalin attenuates lipopolysaccharide-induced inflammatory responses by down-regulating NF-κB signaling pathway**.** *Biochemical and Biophysical Research Communications*, **2012**, 419, 256–261.
18. Awodele, O. I.; Oreagba, A.; Odoma, S.; Silva, J. A.; Osunkalu, V. O. Toxicological evaluation of the aqueous leaf extract of *Moringa oleifera* Lam. (Moringaceae), *Journal Ethnopharmacol*, **2012**, 139, 330–336.
19. Deepak, V.; Kalishwaralal, K.; Ramkumarpandian, S.; Babu, S.V.; Senthilkumar, S.; Sangiliyandi, G. Optimization of media composition for Nattokinase production by *Bacillus subtilis* using response surface methodology, *Bioresource Technology*, **2008**, 99 (17), 8170–8174.
20. Singleton, V.L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybolic – phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, **1965**, 16, 144–158.
21. Brand-Williams, W.,;Cuvelier, M. E., Berset, C. Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology*, **1995**, 28, 1, 25–30.
22. Cheng, Z.; Moore, J.; Yu, L. High-throughput relative DPPH radical scavenging capacity assay, *Journal of Agricultural and Food Chemistry*, **2006**, 54, 7429–7436
23. Sharma, O. P.; Bhat, T. K. DPPH antioxidant assay revisited, *Food Chemistry*, **2009**, 113, 1202–1205.
24. AOAC Official Methods. *Official Methods of Analysis of AOAC International (OMA)*, **2003**, 17th Ed current through 2nd revision.

25. Bligh, E.G.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. Journal Biochemistry Physiology*, **1959**, 3, 911–917.

26. Okawa, H.; Ohishi, N.; Yagi, K. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. *Annals of Biochemistry*. **1979**, 95:351–358.

27. Verardo, V.; Gómez-Caravaca, A. M.; Gori, A.; Losi, G.; Caboni, M. F. Bioactive lipids in the butter production chain from Parmigiano Reggiano cheese area, *Journal of Science and Food Agriculture*, **2013**, 93, 3625–3633.

28. Hartman, L.; Lago, R. C. A. Rapid preparation of fatty acid methyl esters from lipids. *Laboratory Practice*, **1973**, 22, p.475-476.

29. Hough, G.; Pilar-Buera, M.; del, Chirife, J.; Moro, O. O. Sensory texture of commercial biscuits as a function of water activity. *Journal of texture studies*, **2001**, 32, 57-74.

30. Maturin, L. J.; Peeler, J. T.; Bacteriological Analytical Manual Online - of aerobic plate count, *Food and Drug Administration,* **2001**, 8.

31. Shah, M. A; Sowriappan, J. D. B.;, Shabir, A. M. Effect of *Moringa oleifera* leaf extract on the physicochemical properties of modified atmosphere packaged raw beef, *Food Packaging and Shelf Life*, **2015**, 3, 31–38.

32. Das, A. K.; Rajkumar, V.; Verma, A. K.; Swarup, D. *Moringa oleifera* leaves extract: a natural antioxidant for retarding lipid peroxidation in cooked goat meat patties. *International Journal of Food Science and Technology*, **2012**, 47, 585–591.

33. Brazil (**2000**). Ministry of Agriculture, Livestock and Supply. *Normative Instruction*, no. 4, from 31/03/2000. Technical regulation to set the identity and quality of mechanically recovered meat, mortadella, sausage and fresh sausage. Official Journal of the Union. Brasília, DF, April 5, 2000, p. 6–10.

34. Al-Abdullah, B. M; Al-Shuibi, A. M. Substitution of nitrite by sorbate and the effect on properties of mortadella, *Meat Science*, **2002**, 62, 473–478.

35. Greene, B. E.; Cumuze, T. H. Relationship between TBA numbers and inexperienced panelists' assessments of oxidized flavor in cooked beef, *Journal of Food Science*, **1981**, 47, 52–58.

36. Bloukas, J. G.; Paneras, E. D. & Fournitzis, G. D. Effect of replacing pork backfat with olive oil on processing and quality characteristics of fermented sausages. *Meat Science*, **1997**, 45, 2, 133-144.

37. Ferguson, L. R. Role of plant polyphenols in genomic stability. *Mutaion. Research*. **2001**, 475, 89– 111.

38. Jung, J. H.; Choe, B.; Kim, H.; Yun, Z. A.; Kruk, B. Jo, Effect of dietary mixture of gallic acid and linoleic acid on antioxidative potential and quality of breast meat from broilers**,** *Meat Science*, **2010**, 86, 520–526.

39. Yinggang, T.; Sheng, Z.; Mingyong, X.; Weiya, W.; Hongjing, W.; Deming, G.;Composition of fatty acids in the muscle of black-bone silky chicken (*Gallus gellus domesticus brissen*) and its bioactivity in mice, *Food Chemistry*, **2011,** 126, 479–483.

40. Tres, A.; Magrinyà, N.; Bou, R.; Guardiola, F.; Nuchi, C. D.; Codony, R. Impact of the oxidative quality of fish oils in feeds on the composition and oxidative stability of chicken and rabbit meat, *Animal Feed Science and Technology***, 2014,** 196, 76–87.

41. Smith, D. P.; Fletcher, D. L.; Qiao M**.** The relationship of raw broiler breast meat color and pH to cooked meat color and pH, *Poultry Science, Stanford*, **2000**, 79, 788.

42. Herrero, A. M.; Hoz, L.; Ordóñez, J. A.; Herranz, B.; Romero de Ávila, M. D.; Cambero, M. I. Tensile properties of cooked meat sausages and their correlation with texture profile analysis (TPA) parameters and physico-chemical characteristics, *Meat Science***, 2008,** 80, 690–696.

43. COMMISSION REGULATION (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs**.** *Official Journal of the European Union*. **2005**, 338.

44. Radhakrishna, K.; Hygreeva, D.; Pandey, M.C. Potential applications of plant based derivatives as fat replacers, antioxidants and antimicrobials in fresh and processed meat products Review Article, *Meat Science*, **2014**, 98, 459–467.

Figure captions

Figure 1: Optimization surfaces for total phenolic compounds and EC50 activity.

Figure 2: Comparison chart of induction time of samples from different mortadella formulations.

Table 1. Ingredients used in the manufacture of the formulations of mortadella, in percentages of ingredients.

Ingredients	Amounts in formulation (%)			
	CONT	M0.25	M0.50	M1.00
Mechanically deboned chicken meat	45.00	45.00	45.00	45.00
Boneless and skinless chicken breast meat	35.00	35.00	35.00	35.00
Ice/water	12.00	12.00	12.00	12.00
Cassava starch	3.00	3.00	2.75	2.25
Textured soy protein	2.00	2.00	2.00	2.00
Curing salt	0.25	0.25	0.25	0.25
Sodium erythorbate	0.25	0	0	0
Phosphate	0.25	0.25	0.25	0.25
Flavoring for commercial mortadella	0.40	0.40	0.40	0.40
Sodium chloride	1.63	1.63	1.63	1.63
Garlic powder	0.10	0.10	0.10	0.10
White pepper powder	0.02	0.02	0.02	0.02
Monosodium glutamate	0.10	0.10	0.10	0.10
Extract of leaves of *Moringa oleifera*	0	0.25	0.50	1.00

Table 2. Times and correspondent extract solution, with results, for the analysis of total phenolic compounds and EC50.

Time (Hour:min)	Ethanol concentration (% v/v)	Total Phenolic Compounds (mg GAE/L)	Extract concentration (mg de extract/L) 50% DPPH radical sequestration
3:00	50	1080.00	16.38
3:00	0	726.15	11.88
3:00	0	726.15	11.67
3:00	100	51.85	12.57
3:00	100	77.23	10.51
8:25	0	1950.77	30.76
8.25	25	1812.31	29.17
8.25	75	1820.00	37.14
13:50	0	845.69	136.51
13:50	100	680.00	14.83
13:50	50	1072.31	15.82
18:75	25	772.31	12.38
18:75	75	780.00	13.68
24:00	0	856.92	11.69
24:00	0	106.46	15.15
24:00	100	733.85	15.99
24:00	100	726.15	14.99
24:00	50	97.23	15.44
24:00	50	106.46	14.74

Table 3. Centesimal analysis rates of mortadella samples with the addition of *Moringa oleifera* extract.

Sample	Ashes (%)	Humidity (%)	Proteins (%)	Lipids (%)
CONT	3.40 [a] ±0.48	66.42 [a] ±0.18	14.92 [a] ±0.52	10.01 [a] ±0.11
M0.25	3.18 [a] ±0.88	65.50 [a] ±0.05	14.49 [a] ±0.27	9.39 [a] ±0.02
M0.50	3.81 [a] ± 0.55	64.57 [a] ±0.32	13.86 [a] ±0.35	10.63 [a] ±0.41
M1.00	3.09 [a] ±0.30	68.99 [a] ±0.14	12.80 [a] ±0.56	10.36 [a] ±0.12

Table 4. pH and TBARS rates of mortadella samples with the addition of *Moringa oleifera* extract as a function of storage time.

Storage time (days)	pH				TBARs (mg de malonaldehyde /kg)			
	CONT	M0.25	M0.50	M1.00	CONT	M0.25	M0.50	M1.00
0	6.56 [a]	6.70 [a]	6.35 [a]	6.63 [a]	0.13 [a]	0.13 [a]	0.12 [a]	0.14 [a]
15	6.65 [a]	6.53 [a]	6.81 [a]	6.79 [a]	0.10 [a]	0.14 [a]	0.15 [a]	0.16 [a]
30	6.62 [a]	6.75 [a]	6.66 [a]	6.53 [a]	0.17 [a]	0.14 [a]	0.15 [a]	0.13 [a]
45	6.67 [a]	6.36 [a]	6.36 [a]	6.38 [a]	0.17 [a]	0.13 [a]	0.14 [a]	0.19 [a]
60	6.54 [a]	6.42 [a]	6.38 [a]	6.39 [a]	0.17 [a]	0.12 [a]	0.15 [a]	0.18 [a]
75	6.59 [a]	6.71 [a]	6.70 [a]	6.41 [a]	0.17 [a]	0.13 [a]	0.18 [a]	0.20 [a]
90	6.66 [a]	6.48 [a]	6.67 [a]	6.44 [a]	0.18 [a]	0.17 [a]	0.21 [a]	023 [a]
105	6.57 [a]	6.52 [a]	6.52 [a]	6.58 [a]	0.18 [a]	0.20 [a]	0.22 [a]	0.24 [a]
120	6.61 [a]	6.58 [a]	6.49 [a]	6.62 [a]	0.18 [a]	0.23 [a]	0.23 [a]	0.21 [a]
Standard deviation	±0.04	±0.13	±0.16	±0.13	±0.02	±0.03	±0.03	±0.03

Table 5. Oxidation induction points (PI) of mortadella samples from each formulation ($p<0.05$).

Sample	Mean PI (min)
CONT	376.0[a]
M0.25	344.0[b]
M0.50	78.5[c]
M1.00	53.5[d]

Table 6. Concentration of fatty acids in samples of each chicken mortadella formulation, with the addition of *Moringa oleifera* extract.

Fatty Acid	CONT (%)	M0.25 (%)	M0.50 (%)	M1.00 (%)
Myristic (C14:0)	0.54 [a]	0.55 [a]	0.59 [a]	0.56 [a]
Palmitic (C16:0)	22.54 [a]	22.32 [a]	22.70 [a]	22.79 [a]
Palmitoleic (C16:1)	4.00 [a]	4.33 [a]	4.72 [a]	4.40 [a]
Stearic (C18:0)	6.31 [a]	6.20 [a]	6.54 [a]	6.51 [a]
Oleic (C18:1)	34.91 [a]	36.16 [a]	36.65 [a]	35.33 [a]
Linoleic (C18:2)	22.67 [a]	22.71 [a]	24.29 [a]	21.96 [a]
Arachidonic (C20:4)	0.46 [a]	0.42 [a]	0.42 [a]	0.44 [a]
Σ SFA	29.35 [a]	29.07 [a]	29.83 [a]	29.86 [a]
Σ MUFA	61.58 [a]	63.20 [a]	65.66 [a]	61.69 [a]
Σ PUFA	0.46 [a]	0.42 [a]	0.42 [a]	0.44 [a]

Table 7: a *, b * and L * rates in the samples of each chicken mortadella formulation, with addition of Moringa extract.

	a*				b*				L*			
	CONT	M0.25	M0.50	M1.00	CONT	M0.25	M0.50	M1.00	CONT	M0.25	M0.50	M1.00
0	12.98[a]	13.32[a]	13.58[a]	12.87[a]	13.87[a]	14.05[a]	15.26[a]	14.32[a]	58.23[a]	60.28[a]	60.54[a]	58.67[a]
15	12.75[a]	13.57[a]	13.38[a]	12.90[a]	13.74[a]	15.93[a]	16.06[a]	13.36[a]	58.84[a]	61.43[a]	60.90[a]	58.39[a]
30	13.73[a]	12.55[a]	12.63[a]	12.11[a]	14.44[a]	17.51[a]	14.28[a]	13.10[a]	59.07[a]	65.01[a]	59.79[a]	61.59[a]
45	13.30[a]	13.05[a]	11.83[a]	13.12[a]	14.23[a]	14.99[a]	14.57[a]	13.94[a]	59.62[a]	62.34[a]	61.08[a]	61.37[a]
60	12.25[a]	13.82[a]	12.65[a]	13.57[a]	15.00[a]	15.99[a]	15.86[a]	14.83[a]	61.11[a]	58.59[a]	60.98[a]	60.88[a]
75	13.63[a]	12.93[a]	13.01[a]	13.43[a]	14.88[a]	14.02[a]	16.54[a]	15.50[a]	59.65[a]	58.29[a]	62.50[a]	60.58[a]
90	12.74[a]	12.34[a]	13.58[a]	13.78[a]	14.60[a]	15.28[a]	16.24[a]	15.42[a]	59.56[a]	61.33[a]	61.39[a]	58.44[a]
105	12.55[a]	15.06[a]	13.68[a]	12.33[a]	15.95[a]	16.08[a]	16.18[a]	13.91[a]	61.99[a]	59.50[a]	60.89[a]	59.03[a]
120	13.76[a]	14.05[a]	12.04[a]	12.40[a]	14.19[a]	14.57[a]	14.58[a]	15.65[a]	57.86[a]	60.27[a]	64.39[a]	64.88[a]

Table 8. Texture and Aw rates as a function of formulation change ($p < 0.05$).

Sample	Texture (N)	Aw (%)
CONT	10.19[a]	0.965
M0.25	9.66[b]	0.925
M0.50	8.81[c]	0.965
M1.00	7.31[d]	0.927

Means followed by different letters followed by indices in column differ by Tukey´s test ($p < 0.05$).

Table 9: Average scores related to sensory parameters in samples of each chicken mortadella formulation, with the addition of Moringa extract.

Sample	Color	Odor	Flavor	Texture	Overall Appearance
CONT	4.76 [a]	6.05 [a]	7.20 [a]	6.50 [ab]	6.80 [b]
M0.25	6.70 [bc]	6.05 [a]	7.00 [a]	7.75 [b]	6.81 [b]
M0.50	6.27 [b]	6.05 [a]	6.44 [a]	5.25 [a]	6.01 [a]
M1.00	7.06 [c]	6.00 [a]	7.08 [a]	7.29 [b]	7.12 [b]

Means followed by different letters followed by indices in column differ by Tukey´s test ($p < 0.05$).

Table 10: Microbiological analyses during refrigerated storage of mortadella for 120 days.

Storage time (days)	Sample	Thermo-tolerant Coliforms (45°C) (NMP/g)	Total Coliforms (35°C) (NMP/g)	Clostridium sulfite reducer (46°) (UFC/g1)	*Staphylococcus aureus (positive coagulase) (UFC / g)*	*Salmonella spp. (- / +)*	*E. coli (- / +)*
0	CONT	$0.5x10^{2\,a}$	$2.6\ x10^{2\,a}$	$1.9x10^{2\,a}$	<100	Absence	Absence
	M0.25	$2.5x10^{b}$	$2.3\ x10^{b}$	$2.4\ x10^{2\,a}$	<100	Absence	Absence
	M0.50	$2.5x10^{b}$	$2.1\ x10^{b}$	$3.8\ x10^{2\,a}$	<100	Absence	Absence
	M1.00	$2.5x10^{b}$	$2.5\ x10^{b}$	$2.8\ x10^{2\,a}$	<100	Absence	Absence
30	CONT	$2.7x10^{2\,a}$	$2.2\ x10^{2\,a}$	$2.2\ x10^{2\,a}$	<100	Absence	Absence
	M0.25	$2.5x10^{b}$	$2.5\ x10^{b}$	$2.5\ x10^{2\,a}$	<100	Absence	Absence
	M0.50	$1.4x10^{b}$	$2.2\ x10^{b}$	$2.8\ x10^{2\,a}$	<100	Absence	Absence
	M1.00	$2.4x10^{b}$	$2.2\ x10^{b}$	$3.1\ x10^{2\,a}$	<100	Absence	Absence
60	CONT	$2.5x10^{3\,a}$	$1.7\ x10^{3\,a}$	$4.2\ x10^{2\,a}$	<100	Absence	Absence
	M0.25	$2.1x10^{b}$	$2.5\ x10^{b}$	$2.2\ x10^{2\,a}$	<100	Absence	Absence
	M0.50	$2.6x10^{b}$	$1.5\ x10^{b}$	$3.1\ x10^{2\,a}$	<100	Absence	Absence
	M1.00	$2.5x10^{b}$	$2.1\ x10^{b}$	$2.6\ x10^{2\,a}$	<100	Absence	Absence
90	CONT	$2.7x10^{3\,a}$	$2.7\ x10^{3\,a}$	$3.3\ x10^{2\,a}$	<100	Absence	Absence
	M0.25	$2.7x10^{b}$	$2.3\ x10^{b}$	$2.2\ x10^{2\,a}$	<100	Absence	Absence
	M0.50	$2.7x10^{b}$	$2.3\ x10^{b}$	$2.5\ x10^{2\,a}$	<100	Absence	Absence
	M1.00	$2.8x10^{b}$	$2.9\ x10^{b}$	$2.6\ x10^{2\,a}$	<100	Absence	Absence
120	CONT	$2.9x10^{3\,a}$	$2.0\ x10^{3\,a}$	$3.9\ x10^{2\,a}$	<100	Absence	Absence
	M0.25	$2.4x10^{b}$	$2.8\ x10^{b}$	$2.8\ x10^{2\,a}$	<100	Absence	Absence
	M0.50	$2.3x10^{b}$	$2.3x10^{b}$	$2.4\ x10^{2\,a}$	<100	Absence	Absence
	M1.00	$2.7x10^{b}$	2x8x10 ^{b}	$2.3\ x10^{2\,a}$	<100	Absence	Absence
Standard (CE) N.°2073/2005		10^3UFC/g	10^3UFC/g	$5x10^2$UFC/g	$3x10^3$UFC/g	Absence in 10g	$5{,}0x\ 10^3$ UFC/g
Standard deviation		±0.58	±1.00	±1.15	0	0	0

Figure 1.

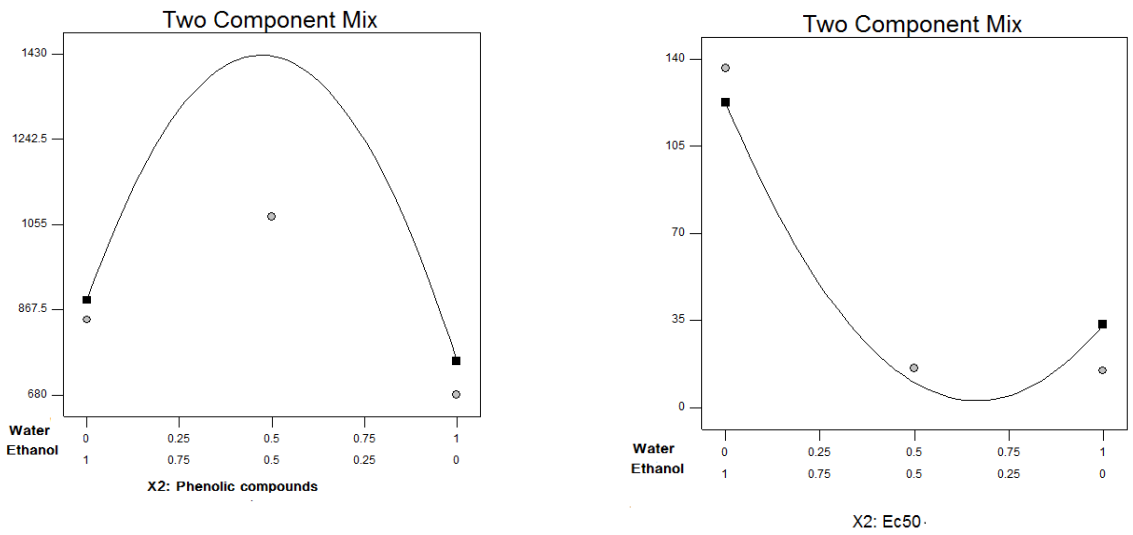

Figure 2.