University „Dunărea de Jos” of Galaţi

Doctoral School of Fundamental Sciences and Engineering

DOCTORAL THESIS

RESEARCH ON THE USE OF VEGETABLE EXTRACTS AND POWDERS AS NATURAL ANTIOXIDANTS IN RAW AND PROCESSED MEAT PRODUCTS

(Doctoral thesis summary)

PhD

Boruzi Andrei Iulian

Scientific leader, Prof univ.dr. NOUR VIOLETA
Scientific reviewers Prof univ.dr. RÂPEANU GABRIELA
Prof univ.dr. POIANĂ MARIANA ATENA
Prof univ.dr. CODINĂ GEORGIANA GABRIELA

Series I 7 Food engineering Nr. 9

GALAŢI

2020

The series of doctoral theses defended publicly in UDJG starting with October 1, 2013 are:

ENGINEERING SCIENCES

- Series I 1: Biotechnologies
- Series I 2: Computers and information technology
- Series I 3. Electrical engineering
- Series I 4: Industrial engineering
- Series I 5: Materials engineering
- Series I 6: Mechanical engineering
- Series I 7: Food Engineering
- Series I 8: Systems Engineering
- Series I 9: Engineering and management in agriculture and rural development

ECONOMIC SCIENCES

- Series E 1: Economy
- Series E 2: Management

HUMANITIES SCIENCES

- Series U 1: Philology - English
- Series U 2: Philology - Romanian
- Series U 3: History
- Series U 4: Philology - French

MATHEMATICS AND NATURAL SCIENCES

- Series C: Chemistry

Table of contents

Keywords: *plant extracts, meat products, lipid oxidation, pH, color, antioxidant activity,* polyphenols, cherry stems, walnut leaves, refrigerated storage, edible films

I. INTRODUCTION

Although meat is a healthy food, having high biological value proteins, a high content of essential minerals and vitamins in group B, it is free of antioxidants. Lipid oxidation is one of the causes of deterioration of meat and meat preparations because it is accompanied by the occurrence of a large number of unwanted changes in flavor, texture and nutritional value. The speed of lipid oxidation can be effectively reduced by the use of antioxidants. Synthetic antioxidants have been widely used in the meat industry, but consumer concerns about product safety and toxicity have led the food industry to seek natural alternatives. As a result, some natural ingredients, including herbs and spices, have been studied, especially in Asian countries, as potential antioxidant products in meat and meat products. Research has shown that natural antioxidants extracted from plants, such as rosemary, sage, tea, soy, citrus peel, sesame seeds, olives, tomatoes and grapes, can be used as alternatives to synthetic antioxidants because of their equivalent or even greater effect. on inhibition of lipid oxidation. Herbal and spice compounds contain many phytochemicals that are potential sources of natural antioxidants including phenolic diterpenes, flavonoids, tannins and phenolic acids. These compounds have antioxidant, anti-inflammatory and anti-carcinogenic activities. In food systems, they can enhance the aroma, delay food damage induced by lipid oxidation, inhibit the growth of microorganisms, and play roles in reducing the risk of diseases. These few considerations highlight the opportunity and importance of studies related to the enrichment of meat and meat products with bioactive compounds with health benefits through the incorporation of powders and plant extracts.

The studies developed in this paper started from the hypothesis that plant extracts, with a high content of specific phytochemicals, obtained from various plant materials, will have synergistic antioxidant and antimicrobial effects, inhibit the growth of pathogenic and altering bacteria and the evolution of oxidative processes. in different meat products and will improve the quality and safety of the meat. It was also hypothesized that the incorporation of complex plant extracts with antioxidant properties in processed meat products will result in healthier products due to the lower oxidation level of the meat, thus preventing inflammatory reactions, without significantly affecting the sensory characteristics. of them. As a result, the studies developed within this doctoral thesis had as main objective the improvement of the functional value of meat products by the addition of powders and natural extracts with antioxidant properties.

Walnut leaves and cherry stems have been studied because they are recognized for their therapeutic properties, their content in biologically active compounds and high antioxidant activity.

In the context of current research, the doctoral thesis proposed the following specific scientific objectives:

→ Obtaining and characterizing plant extracts and powders for use in raw and processed meat products;

→ Study of the effects of incorporating powders and extracts from walnut leaves and cherry stems on physical-chemical and sensory characteristics of cooked pork patties;

→ Evaluation of antimicrobial activity of walnut leaf powder and extracts of walnut leaves and cherry stems in cooked pork patties;

→ Studying the effectiveness of walnuts and cherry stems as natural antioxidants in raw minced pork during frozen storage;

→ Effects of chitosan film-coated film enriched with walnut leaf extracts and cherry stems on the quality of precooked pork patties.

II. DOCUMENTARY RESEARCH

CHAPTER 1

THE IMPACT OF MEAT CONSUMPTION ON HUMAN HEALTH

Today, consumers are giving more and more importance to all aspects that can contribute to improving the quality of life and diet, although not the only element that influences well-being and health, is one of the most important. Factors that have fostered this development include the current extremely high impact on public opinion of media reports on the relationship between diet and health, increasing the life expectancy of the population (this generates high purchasing power consumers who have higher health problems and are highly motivated to participate in initiatives to maintain health), increased attention to disease prevention etc. This situation is behind the spectacular development of "healthy" products, ie products that must have one of the following characteristics: modified composition and / or processing conditions so as to prevent or limit the presence of certain potentially harmful compounds and / or the possibility of include certain desired substances, which have additional health benefits, either naturally or by addition. This concept also includes those foods known as "functional foods". They are defined as foods that help prevent and treat diseases and illnesses, in addition to their nutritional value per se. In fact, this is not a new idea, for centuries mankind has used the properties of food to treat, alleviate or prevent disease. However, the large amount of scientific evidence indicating the relationship between food consumption and disease incidence has generated a growing interest in foods that provide additional physiological benefits.

Meat and meat products are important sources of protein, vitamins and minerals, but also contribute to the intake of fats, saturated fatty acids, cholesterol, salt, etc. In order to produce "healthier" meat products, we need to have a good understanding of their health advantages and disadvantages [1].

Like all other foods, meat and meat products contain elements that, under certain conditions and in inappropriate proportions, can adversely affect human health. Some of these are constituents (natural or other) present in live animals, for example fats, cholesterol, residues from environmental pollution or the use of pharmaceuticals. etc. Others are added to the product during processing for technological, microbiological or sensory reasons (salt, nitrite, phosphate, etc.). There is a third group that is produced by the technological treatment used (including contaminants from disinfectants or detergents, toxic compounds formed during processing, etc.). Finally, they are those that are formed especially in the storage / marketing phase, such as the development of pathogenic bacteria, the formation of certain lipid oxidation products and the migration of compounds from the packaging material into the product.

CHAPTER 2

OXIDATION OF LIPIDS IN MEAT AND MEAT PRODUCTS

Lipid oxidation is the main cause of deterioration of meat quality during storage and processing [2]. Primary and secondary oxidation products alter flavor, color, texture and decrease nutritional quality [3].

Lipid oxidation is a very complex process, initiated by the peroxidation of unsaturated fatty acids from phospholipid membranes, to form primary oxidation products, hydroxyperoxides. They decompose to form by-products of oxidation, such as aldehydes, ketones, alkenes and alcohols, which cause unpleasant odors which adversely affect the acceptability and overall quality of meat and meat products [4]. Reactions responsible for oxidation of myoglobin and lipids generate products that can in turn act to accelerate oxidation [5]. Therefore, antioxidants are added to maintain the quality and shelf life of meat and other fat-rich products. Antioxidants are able to stabilize free radicals by cloning hydrogen (H) to free radicals or by accepting electrons from free radicals to form complexes [6]. Antioxidants are often consumed during meat processing and storage. Thus, adding them to the final product is a strategy used to minimize damage during storage and, consequently, to increase the shelf life of the product [4]. Synthetic antioxidants have been widely used to minimize the oxidation of lipids in food. However, due to the growing concern about the safety of synthetic chemicals, the use of natural compounds with antioxidant activity is preferred and has attracted the attention of researchers [7, 8, 9]. As a result, more attention has been paid to research into natural substances that can act as alternative antioxidants. The addition of natural antioxidants from plant extracts as a way to increase the shelf life of foods has become increasingly popular. Also, their use has improved the stability of lipids and lipid-containing foods, thus preventing the loss of sensory and nutritional quality [10, 11, 12].

Research on natural antioxidants has intensified in recent years; These antioxidants can be found in any part of the plant, such as grains, fruits, nuts, seeds, leaves, roots and bark. Most natural antioxidants are phenolic compounds, and the most important are tocopherols, flavonoids and phenolic acids. All are generally common to all plant sources. They are added to a wide variety of foods to prevent or delay the oxidation of lipids.

Phenolic compounds present in natural antioxidants have a strong $H^{\cdot}$ cloning activity or have a high capacity to absorb radicals [13]. The main antioxidant phenolic compounds are: phenolic acids, phenolic diterpenes, flavonoids and volatile oils. Some phenolic compounds prevent the formation of free radicals and the spread of ROS, while others eliminate free radicals and chelate prooxidants (transition metals) [14]. Phenolic acids capture free radicals, flavonoids eliminate free radicals and chelate metals (Fe^{2+}, Fe^{3+} și Cu^{2+}). The antioxidant potential of these natural compounds (phenolic compounds) depends on the structure of their skeleton and the functional groups on this skeleton [15]. For example, the number and location of free hydroxyl (–OH) groups on the flavonoid backbone determine the potential for free radical scavenging [16]. The presence of several –OH groups and ortho-3,4-dihydroxy structures increase the antioxidant potential of phenolic compounds derived from plants [17, 18].

CHAPTER 3

IMPROVING THE FUNCTIONAL VALUE OF MEAT PRODUCTS BY ADDING NATURAL EXTRACTS WITH ANTIOXIDANT PROPERTIES

3.1. Incorporation of functional ingredients in meat and meat products

Increasing consumer concerns about the health and physiological effects of food or food components on it, and the tendency to consume functional foods have led to the need to provide meat and meat products with a number of health-beneficial properties and even to change their image among consumers. The inclusion of functional ingredients in meat and meat preparations aims not only to provide certain desired properties but also to try to change their unfortunate image among consumers. This image is mainly determined by the content of fat, saturated fatty acids and cholesterol and their association with cardiovascular disease, some cancers, obesity and others.

The changes to which meat can be subjected to give it functional properties are based on changes in animal feed or post-mortem handling of carcases. As for the first way, it can lead to a change in the content of lipids, fatty acids and vitamin E in the meat, while in the second way, the fat can be changed by mechanical processes.

With regard to meat preparations, efforts are aimed in particular at reformulating them by changing the content of lipids and fatty acids and / or by adding functional ingredients (fibers, vegetable proteins, monounsaturated or polyunsaturated fatty acids, vitamins, calcium, phytochemicals, etc.).

3.2. Use of natural antioxidants in meat and meat products

Antioxidants from natural sources offer a good alternative to conventional antioxidants, due to the high content of phenolic compounds and other active ingredients, which can effectively prevent the initiation or spread of lipid oxidation reactions. Some of these antioxidants have been shown to have stronger antioxidant properties than BHA / BHT. The results obtained in these studies showed that various natural antioxidants exert positive or negative effects on the color and sensory properties of meat products.

Natural antioxidants extracted from plants can be used as alternatives to synthetic antioxidants due to their equivalent or even greater effect on inhibiting lipid oxidation [19].

Numerous studies have shown that these natural antioxidants have been very effective in preventing the oxidation of lipids, compared to a certain positive control (BHA / BHT), in different storage conditions.

CHAPTER 4

EDIBLE FILMS AND COATINGS FOR IMPROVING THE QUALITY OF MEAT AND MEAT PRODUCTS

4.1. Use of edible films and coatings in the meat industry

Coating food with edible materials has been proposed and studied as an effective method of improving food quality [20].

Edible / biodegradable coatings and films produced from polysaccharides, proteins and / or waxes and lipid derivatives may function as effective barriers against moisture and / or oxygen. Edible coatings are also environmentally friendly, avoiding the negative effects caused by non-renewable packaging materials [21, 22, 23]. Therefore, they may be alternative packaging methods that can be used to maintain the quality and extend the shelf life of foods.

Different types of coatings and films have been tested in an attempt to maintain the quality of meat products. Published studies have shown that edible coatings can substantially improve the quality of meat and can be used as an encapsulation matrix of bioactive compounds, allowing their controlled release. Through this strategy, bioactive compounds are made available at a desired place and time at a specific rate [24]. This application is an interesting tool not only to extend the shelf life and reduce the risk of developing pathogens on food surfaces, but also to provide the consumer with a functional product with health benefits.

Starch, alginate, dextrins, pectin, chitosan and carrageenans are used in edible films and coatings. Water-soluble polysaccharides are long-chain polymers that are commonly used in food for their thickening and / or gelling properties [25, 26, 27]. Polysaccharide films are good barriers to gases and are resistant to fats and oils; however, their hydrophilic nature makes them weak barriers to water vapor [28]. They have been used to extend the shelf life of meat products by delaying dehydration, oxidative rancidity and surface browning.

4.2. Edible films and coatings based on chitosan

Chitosan is a high molecular weight cationic polysaccharide, which exhibits antibacterial activity [29, 30] and antifungal activity [31], as well as film-forming properties [32, 33]. Numerous reports have been reported on the potential of chitosan to act as a food preservative, a function that has been assessed on the basis of in vitro studies or by the direct application of chitosan to food [34, 35, 36, 37, 38, 39, 40].

Due to the good film-forming capacity of chitosan, it has been used extensively to protect, improve quality and extend the shelf life of fresh and processed foods. However, the available reports on the application of chitosan and pectin films and the use of chitosan coatings on meat products are however limited.

III. EXPERIMENTAL RESULTS

CHAPTER 5

OBTAINING AND CHARACTERIZATION OF VEGETABLE EXTRACTS AND POWDERS FOR USAGE IN RAW AND PROCESSED MEAT PRODUCTS

5.1. Study Opportunity

Walnut (Juglans regia L.) contains significant amounts of phenolic compounds [41]. It is known that the antioxidant activity is stronger the higher the polyphenol content. Various scientific papers have demonstrated the antioxidant potential of walnut products, respectively walnut kernels [41, 42], green shells [43], leaves [44, 45, 46]. Walnut leaves have been used in both the cosmetic and pharmaceutical industries. Previous studies have shown that both the fruits and leaves of Romanian walnut varieties have proven to be an important source of phenolic compounds [47, 48 , 49].

Infusions and decoctions prepared from cherry stems (Prunus avium L.) are traditionally used as sedatives, diuretics and for drainage [50, 51], contributing in particular to the promotion of adequate renal function. The anti-inflammatory and diuretic properties are correlated with the high content of natural antioxidants, respectively phenolic compounds (phenolic acids and flavonoids) [52], stems being a good candidate for nutraceuticals or pharmaceuticals [53]. The determination of phenolic compounds in walnut leaves and cherry stems is important both for their characterization and for facilitating the more efficient use of these important plant resources.

5.2. Materials and methods of analysis

Fresh walnut leaves (Juglans regia L.) and cherry stems (Prunus avium L.) were collected from trees grown in the experimental orchard of the University of Craiova, located at the Râmnicu Vâlcea Research Station (Romania) (45°07'N/24°22'E). The samples were washed and dried in the shade. Walnut leaves and dried cherry stems were ground up into a fine powder. Walnut leaves and dehydrated cherry stems were analyzed for the total content of phenolic compounds, flavonoids, antioxidant activity and phenolic profile.

The total content of phenolic compounds in the extracts of walnut leaves and cherry stems was determined colorimetrically with Folin-Ciocâlteu reagent.

The total flavonoid content was determined by the aluminum chloride colorimetric method and the results were expressed in mg.

The antioxidant activity of the samples was measured using an ABTS procedure (2,29-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) and the results were expressed in mM Trolox / 100 g. The phenolic profile was determined by reverse phase HPLC with diode-array detection.

All analyzes were performed in triplicate and the results were expressed as mean ± standard deviation (SD). The data were subjected to analysis of variance (ANOVA), and the significance of the differences between the means was assessed with the Duncan multiple comparison test at $p < 0,05$.

5.3. Results and discussion

The total content of phenolic compounds, the total content of flavonoids and antioxidant activity

In the walnut leaf extract, the total flavonoid content and antioxidant activity were higher than in the cherry stem extract. A similar situation was observed in the case of powders. Walnut leaves have been previously reported as a rich source of flavonoids [54, 55]. However, the total content of phenolic compounds was higher in the cherry stem extract and powder than in the walnut leaf extract and powder, respectively.

*Table 5.1. Total content of phenolic compounds, total content of flavonoids and antioxidant activity in extracts and powders of walnut leaves and cherry stems**

Sample	Total content of phenolic compounds (mg GAE/100 g DW)	Total flavonoid content (mg QE/100 g DW)	ABTS antioxidant activity (mmol Trolox/100 g DW)
Walnut leaf powder	2898,28 ± 128,56	1956,76 ± 87,55	16,28 ± 0,87
Cherry stem powder	3334,56 ± 146,21	862,27 ± 48,96	14,62 ± 0,66
Walnut leaf extract	497,67 ± 23,34	353,66 ± 16,45	3,67 ± 0,21
Cherry stem extract	685,66 ± 31,15	145,35 ± 11,19	2,84 ± 0,14

* Values are expressed as mean ± standard deviation.

Seasonal variation of the total content of phenolic compounds in walnut leaves

The total content of phenolic compounds was determined in the dehydrated walnut leaves at eight sampling dates (from 1 June to 15 September) and the results are shown in Table 5.2. Statistically significant differences were obtained between the phenolic compounds content of the leaves harvested at different times ($p < 0,05$).

Table 5.2. Seasonal variation of the total phenolic compounds content of walnut leaves

Date of sampling	Total content of phenolic compounds (mg/g)
1 June	13,47 ± 0,33
15 June	12,77 ± 0,34
1 July	19,68 ± 0,43
15 July	30,77 ± 0,89
1 August	20,75 ± 0,77
15 August	21,44 ± 0,91
1 September	24,67 ± 0,56
1 September	24,21 ± 0,62
Average	21,95 ± 1,07

* Values are expressed as mean ± standard deviation

The total content of phenolic compounds increased in June and July, decreased in August, after which there was a new increase until the beginning of September. Values ranged from 13,47 mg GAE / g (June 1) to 30,77 mg GAE / g (July 15) (Table 5.2). Previous studies have revealed a considerable variation depending on the variety [43] and also highlighted the seasonal variation in the content of phenolic compounds in walnut leaves [47, 56, 57]. These variations are supposed to be the effect of changing environmental conditions.

Phenolic profile of walnut leaves and cherry stems

A chromatogram of the walnut leaf extract is shown in Figure 5.1.

The results on the phenolic acid content showed that ellagic acid was the most abundant phenolic acid in all samples, followed by trans-cinnamic and chlorogenic acids. In lower concentrations, caffeic, ferulic, synaptic, salicylic, syringic, p-coumaric, gallic and vanillic acids were determined in walnut leaves.

Juglona is known to be the characteristic compound of Juglans spp. And has been determined in a number of studies in fresh walnut leaves [49, 56]. Lately, interest in juglon has increased, as several studies have shown that juglon exerts cytotoxic and genotoxic effects against tumor cells, significantly reducing their proliferation [58].

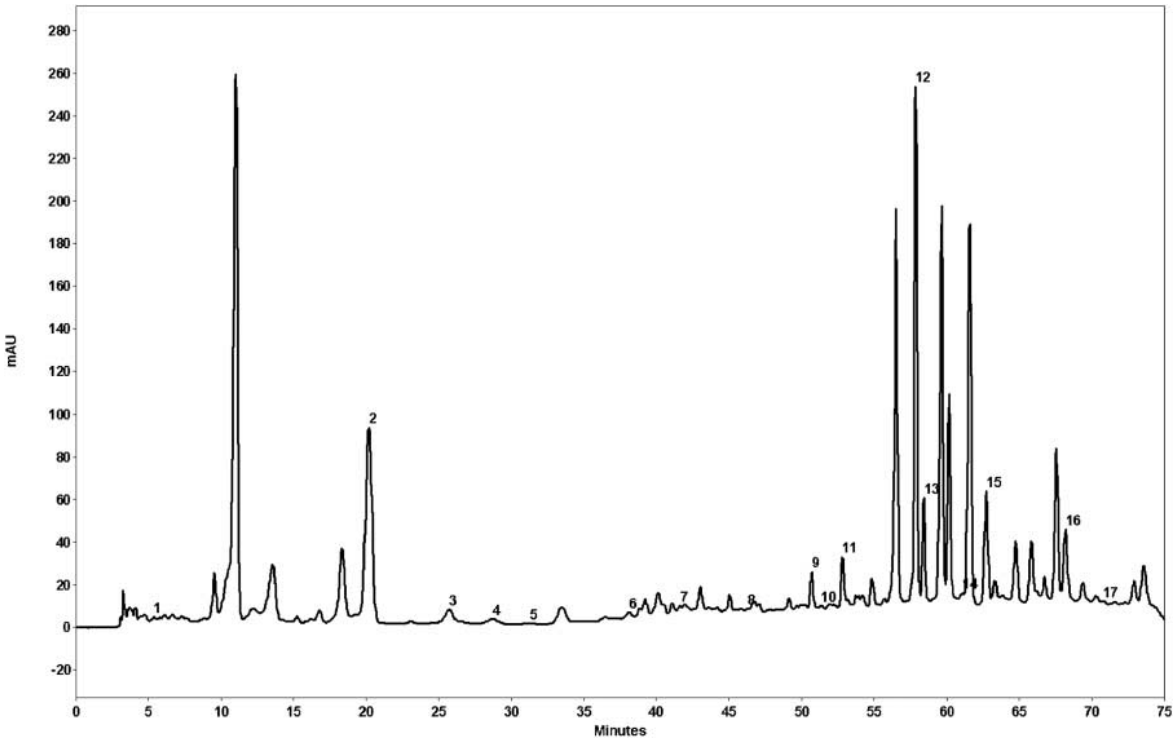


Figure 5.1. Chromatogram of walnut leaf extract at λ=278 nm: 1, gallic acid; 2, catechin-hydrate; 3, vanillic acid; 4, chlorogenic acid; 5, caffeic acid; 6, syringic acid; 7, epicatechin; 8, p-coumaric acid; 9, ferulic acid; 10, sinapic acid; 11, salicylic acid; 12, rutin; 13, ellagic acid; 14, myricetin; 15, juglone; 16, trans-cinnamic acid; 17, quercetin.

5.4. Partial conclusions

From the results obtained it can be concluded that the powders and extracts of walnut leaves and cherry stems have a high content of phenolic compounds and a high antioxidant activity, as a result they can be extremely valuable sources of phenolic compounds and can be used as alternative natural antioxidants.

Table 5.3. The content of phenolic compounds in walnut leaves and cherry stems

Compound	Content (mg/100 g FW)	
	Walnut leaves	Cherry stems
Vanillic acid	0,03 ± 0,001	0,11 ± 0,01
Rutin	78,04 ± 3,61	56,67 ± 0,65

Elagic acid	67,34 ± 2,37	33,77 ± 1,10
Myricetin	91,71 ± 1,67	10,64 ± 0,48
Juglone	48,23 ± 2,45	ud*
Quercetin	3,11 ± 0,07	6,32 ± 0,29
Gallic acid	0,39 ± 0,03	4,33 ± 0,19
Catechin-hydrate	304,93 ± 4,58	247,66 ± 7,18
Syringic acid	0,76 ± 0,02	2,91 ± 0,14
Epicatechin	2,17 ± 0,03	1,87 ± 0,09
Trans-cinnamic acid	9,58 ± 0,33	44,56 ± 2,09
Chlorogenic acid	4,51 ± 0,19	11.88 ± 0,44
Caffeic acid	0,15 ± 0,01	9.11 ± 0,39
P-coumaric acid	0,42 ± 0,02	16.77 ± 0,78
Ferulic acid	1,56 ± 0,07	5,34 ± 0,26
Synaptic acid	1,18 ± 0,04	20,42 ± 0,55
Salicylic acid	1,80 ± 0,04	0,24± 0,02

*ud = undetected

It can also be concluded that there is a dependence between the phenolic content of walnut leaves and seasonal, genetic and ecological factors. The results suggest that, in order to be used as sources of natural antioxidants, walnut leaves should be collected preferentially in July and early September, when the phenolic content is higher.

The information obtained by analyzing the seasonal variation of the total content of phenolic compounds in walnut leaves could be useful in planning the collection time of walnut products to obtain biologically active extracts.

Experimental results indicated that fresh walnut leaves contain high concentrations of juglon, comparable to those reported in previous studies. Based on the results, ellagic acid was established as the dominant phenolic acid in walnut leaves. Although other phenolic acids are present in much lower concentrations, they can greatly contribute to the antioxidant activity of walnut leaves. Flavonoid determinations revealed high concentrations of myricetin, catechin hydrate and rutin and low concentrations of quercetin and epicatechin as aglycones. In cherry stems, trans-cinnamic, synaptic and p-coumaric acids predominated among phenolic acids, while high concentrations of catechin hydrate and rutin were determined between flavonoids.

Although they show lower values of the total content of phenolic compounds, the powder and the extract of the walnut leaf had a higher antioxidant activity, probably due to the higher content of flavonoids than the cherry stems extract.

CAPITOLUL 6

THE INFLUENCE OF THE ADDITION OF WALNUT LEAF POWDER ON SOME PHYSICO-CHEMICAL AND SENSORY CHARACTERISTICS OF MINCED COOKED PORK MEAT AND ON THEIR EVOLUTION DURING STORAGE

6.1. Study Opportunity

In this experiment, walnut leaf powder was used as a source of natural antioxidants in pork patties. Its antioxidant activity in the meat product was evaluated in relation to butylhydroxytoluene at an addition level of 0,1% and in relation to a control without antioxidant addition. Therefore, this study aims to assess the oxidative stability and color stability of pre-cooked minced pork patties containing walnut leaf powder at addition levels of 0,2% (P1) and 0,5% (P2), compared to a control without addition of antioxidant (M0) and a control with addition of 0,1% butylhydroxytoluene (BHT) (M1).

6.2. Materials and methods of analysis

The samples of minced meat (patties) were produced using the following recipe: 73,5% lean pork meat, 20% back fat, 5% ice and 1,5% salt. The lean pork meat and back fat were chopped through a sieve with 3 mm holes, then ice and salt were added. The mixture was divided into four experimental variants. Thus, walnut leaf powder was added at 0% (control without addition of antioxidant - M0), 0,2% (P1), 0,5% (P2) and BHT at 0,1% (control with the addition of antioxidant - M1) in relation to the mass of minced meat without antioxidant powder. The samples were hand-kneaded for 10 minutes. Then, the mixed meat was weighed (50 g) and the samples were formed in a Petri dish resulting in 40 patties / experimental variant. The samples were placed in a preheated electric oven for 15 minutes at 180°C and left to bake for 10 minutes until the meat reached 75 ± 1°C in the center. After removing from the oven, 5 patties were randomly weighed from each batch to determine the quantitative losses during baking. The patties were cooled to room temperature, aerobically packed in polyethylene bags and stored at 4 ± 1°C for 15 days. The baking yield was determined by dividing the mass of the cooked product by the mass of the raw meat mixture and was expressed as a percentage. At baseline and after 5, 10, and 15 days, respectively, samples were analyzed for sensory, color, total phenolic compounds content, pH, ABTS antioxidant activity, and lipid peroxidation using test values. TBARS (ThioBarbituric Acid Reactive Substances).

Color changes during storage were monitored by evaluating CIELab color parameters (L, a, b, Hue and Chroma) at a 5-day interval for 15 days.

All determinations were performed in triplicate and the results were reported as mean ± standard deviation. The data were subjected to analysis of variance (ANOVA) which was used to assess the effect of additions and retention time. The significance of the differences between the means was assessed using the Duncan multiple test at $P < 0,05$. Statistical data analysis was performed using the Statgraphics Centurion XVI software (StatPoint Technologies, VA, USA).

6.3. Results and discussion

The mixture of minced meat originally made from lean pork meat and back fat had an average content of 60,08% moisture, 18,32% protein and 14,51% fat.

The moisture, protein and fat content of heat-processed patties made according to the experimental variants is presented in Table 6.1.

Table 6.1. The moisture, protein and fat content of heat-processed patties

Tratament	Umiditate (%)	Proteine (%)	Grăsimi (%)	Randament la coacere (%)
M0	$51,77 \pm 0,59^{a}$	$22,14 \pm 0,30^{b}$	$17,03 \pm 0,22^{b}$	$78,83 \pm 0,55^{a}$
M1	$52,26 \pm 0,38^{ab}$	$21,97 \pm 0,48^{ab}$	$16,86 \pm 0,38^{ab}$	$79,61 \pm 0,84^{a}$
P1	$53,20 \pm 0,47^{bc}$	$21,68 \pm 0,22^{ab}$	$16,52 \pm 0,34^{ab}$	$81,23 \pm 0,48^{b}$
P2	$53,65 \pm 0,63^{c}$	$21,47 \pm 0,35^{a}$	$16,35 \pm 0,28^{a}$	$82,02 \pm 0,71^{b}$

* the averages in the same column accompanied by different letters to the exponent differ significantly ($P < 0,05$)

The moisture content was higher in the samples to which walnut leaf powder was added indicating that it contributed to the moisture retention in the product. These results correlate with those on the ripening yield, which was significantly higher in the samples with the addition of walnut leaf powder. There were no significant differences between the control samples and those with the addition of BHT in terms of moisture, protein and fat content. The highest protein and fat content was recorded in the control sample, probably as a result of the higher moisture loss in this experimental variant.

The pH variation in pork patties during refrigeration is shown in table 6.2.

Tablel 6.2. pH of samples of control patties and containing walnut leaf powder during storage in a refrigerated state for 15 days

	Refrigerated storage period (days)			
Treatment	0	5	10	15
M0	$6,00 \pm 0,03^{a}$	$6,04 \pm 0,03$	$5,93 \pm 0,06$	$5,85 \pm 0,05^{a}$
M1	$6,11 \pm 0,03^{b}$	$6,04 \pm 0,02$	$5,95 \pm 0,03$	$5,97 \pm 0,05^{b}$
P1	$6,03 \pm 0,07^{ab}$	$6,06 \pm 0,05$	$5,98 \pm 0,03$	$6,06 \pm 0,07^{bc}$
P2	$6,09 \pm 0,06^{ab}$	$6,03 \pm 0,02$	$5,97 \pm 0,05$	$6,05 \pm 0,04^{c}$

* the averages in the same column accompanied by different letters to the exponent differ significantly ($P < 0,05$)

PH values ranged from 5,85 to 6,15 during the storage period.

Although there were no significant differences between the pH values of the different experimental variants during the first 10 days of storage, after 15 days the pH recorded the lowest values in the control sample, while the highest values were recorded in the sample with the addition of 0.5% walnut leaf powder.

During the storage period, the pH level decreased in the control sample and in the sample with the addition of BHT, while in the samples with the addition of walnut leaf powder the pH decreased in the first 10 days after which it began to increase, without the pH values after 15 days of storage differing significantly from those initially recorded. The increase in the

pH of pork patties during storage is due to the exposure of the basic groups by denaturing proteins. Also, the increase in pH is associated with the degradation of proteins and amino acids by gram-negative bacteria [59].

The values of the color parameters (L*, a* and b*) as well as the hue (h) and saturation (C) of the samples of control patties and containing walnut leaf powder and their variation during storage in the refrigerated state for 15 days are presented in table 6.3.

Table 6.3. Values of color parameters of samples of control patties and containing walnut leaf powder during storage in a refrigerated state for 15 days

Color parameters	Treatment	Refrigerated storage period (days)			
		0	5	10	15
L*	M0	76,00 ± 1,39^{b}	75,85 ± 1,18^{c}	74,04 ± 0,39^{c}	76,73 ± 2,00^{c}
	M1	75,04 ± 1,08^{b}	75,67 ± 0,97^{bc}	73,26 ± 2,03^{c}	75,18 ± 0,64^{c}
	P1	74,18 ± 1,40^{ab}	73,93 ± 1,33^{b}	70,25 ± 0,98^{b}	69,80 ± 2,44^{b}
	P2	72,23 ± 1,86^{a}	70,60 ± 1,17^{a}	65,27 ± 1,68^{a}	65,51 ± 0,37^{a}
a*	M0	5,32 ± 0,19^{b}	4,49 ± 0,15^{b}	4,55 ± 0,28^{b}	3,97 ±0,04^{b}
	M1	6,82 ± 0,22^{c}	6,16 ± 0,09^{c}	5,82 ± 0,05^{c}	5,40 ± 0,10^{c}
	P1	5,26 ± 0,46^{b}	4,54 ± 0,44^{b}	4,75 ± 0,39^{b}	4,16 ± 0,30^{b}
	P2	4,25 ± 0,24^{a}	3,85 ± 0,37^{a}	3,88 ± 0,24^{a}	3,49 ± 0,07^{a}
b*	M0	13,10 ± 0,35^{a}	13,84 ± 0,14^{b}	14,27 ± 0,41^{c}	13,96 ± 0,28^{c}
	M1	12,87 ± 0,53^{a}	13,42 ± 0,20^{b}	13,37 ± 0,11^{b}	13,21 ± 0,16^{b}
	P1	13,19 ± 0,31^{a}	12,11 ± 0,30^{a}	12,64 ± 0,62^{ab}	12,25 ± 0,57^{a}
	P2	13,27 ± 0,52^{a}	12,21 ± 0,54^{a}	12,24 ± 0,66^{a}	12,06 ± 0,05^{a}
C	M0	14,14 ± 0,39^{a}	14,55 ± 0,13^{b}	14,98 ± 0,47^{b}	14,51 ± 0,28^{b}
	M1	14,57 ± 0,58^{a}	14,76 ± 0,21^{b}	14,60 ± 0,07^{b}	14,27 ± 0,18^{b}
	P1	14,20 ± 0,46^{a}	12,93 ± 0,42^{a}	13,50 ± 0,71^{a}	12,94 ± 0,62^{a}
	P2	13,93 ± 0,57^{a}	12,81 ± 0,62^{a}	12,84 ± 0,69^{a}	12,56 ± 0,03^{a}
h	M0	67,89 ± 0,20^{b}	72,03 ± 0,64^{c}	72,33 ± 0,71^{c}	74,14 ± 0,15^{c}
	M1	62,07 ± 0,25^{a}	65,35 ± 0,21^{a}	66,53 ± 0,11^{a}	67,75 ± 0,16^{a}
	P1	68,28 ± 1,27^{b}	69,47 ± 1,45^{b}	69,42 ± 0,77^{b}	71,25 ± 0,72^{b}
	P2	72,25 ± 0,30^{c}	72,56 ± 0,94^{c}	72,44 ± 0,53^{c}	73,87 ± 0,37^{c}

*the averages in the same column for the same color parameter accompanied by different letters to the exponent differ significantly (P < 0,05)

The color of the meat depends on the concentration of meat pigments but also on the physico-chemical properties of the meat substances and those added to it. The result of the color evaluation revealed a significant effect (P <0,05) of the addition of walnut leaf powder as a natural antioxidant on the color parameters.

The mean brightness values (L*) at baseline were significantly higher in the control samples than in the samples with the addition of walnut leaf powder, so the addition of walnut leaf powder resulted in the darkening of the pork patties. Also, at this time there were no significant differences in the brightness of the control and the control with the addition of BHT. The increase in the concentration of powder in walnut leaves caused the value of the brightness of the samples of pork patties to decrease. Also, the a* values were significantly higher in the sample with the addition of BHT compared to the control and significantly lower in

the samples with the addition of walnut leaf powder, which can be explained by its green color. In fact, the a* values were significantly lower at P2 compared to P1 due to the higher content of walnut leaf powder.

Regarding the color variation during storage, there is a maintenance or even a slight increase in brightness values (L*) in the control sample and the sample with the addition of BHT, while in the samples with the addition of walnut leaf powder there was a decrease in L values (darkening), more important in sample P2 (0,5% walnut leaf powder). Previous studies have shown that meat discoloration is caused by oxidative processes and reducing enzyme systems [60]. As a result, it can be assumed that, in the control sample, the higher intensity of the oxidative processes led to the observed discoloration of the samples.

Also, the values of a* registered constant decreases during the storage period for all experimental variants. A decrease in a* may also indicate a change in color from red to brown, which may be due to the formation of metmioglobin in salt-containing samples. Salt greatly accelerates the process of discoloration of meat due to the pro-oxidative activity that can be attributed to its ability to release iron from hemic pigments and other molecules that bind hem [61].

It should be noted that the decrease rate of parameter a* was lower in the sample with the addition of 0,5% walnut leaf powder compared to the control, which may be an indicator of the antioxidant activity of walnut leaves and its role. effective as a food ingredient.

The values of the parameter b* decreased during storage in the samples with the addition of powder from walnut leaves but increased in the control sample while the hue values increased during storage in all experimental variants, with the highest rate increase in control samples.

Lipid oxidation is characterized by the formation of conjugated dienes, hydroperoxides and aldehydes [62]. The results presented in Table 6.4 show that lipid oxidation, measured by TBARS values, was significantly delayed (P <0,05) in pork patties during their refrigerated storage by the addition of both BHT and walnut leaf powder. in relation to the witness. TBARS values ranged from 0,06 to 3,37 MDA mg / kg within 15 days at 4°C.

Table 6.4. TBARS values (mg MDA / kg) of samples of control patties and containing walnut leaf powder during storage in a refrigerated state for 15 days

	Refrigerated storage period (days)			
Treatment	0	5	10	15
M0	$2,81 \pm 0,26^{c}$	$3,02 \pm 0,34^{d}$	$3,28 \pm 0,29^{d}$	$3,37 \pm 0,35^{d}$
M1	$0,31 \pm 0,04^{ab}$	$1,36 \pm 0,23^{c}$	$1,74 \pm 0,22^{c}$	$2,06 \pm 0,19^{c}$
P1	$0,32 \pm 0,06^{b}$	$0,82 \pm 0,11^{b}$	$1,23 \pm 0,16^{b}$	$1,34 \pm 0,09^{b}$
P2	$0,06 \pm 0,02^{a}$	$0,07 \pm 0,02^{a}$	$0,13 \pm 0,02^{a}$	$0,15 \pm 0,03^{a}$

* the averages in the same column accompanied by different letters to the exponent differ significantly (P < 0,05)

According to Al-Kahtani [63], meat products can be considered well preserved in terms of oxidative changes, when their TBARS values are below 3 mg MDA / kg. In the present experiment, only the control had values higher than 3 mg MDA / kg, indicating that the control samples showed advanced oxidation and were unfit for consumption.

The TBARS values of patties made with the addition of walnut leaf powder were significantly lower than those without the addition. Initially, the TBARS values of the samples

with 0,2% walnut leaf powder were comparable to those of the samples made with the addition of 0,1% BHT, while the TBARS values of the samples made with the addition of 0,5% Walnut leaf powder were much lower.

Regarding the evolution during the storage of patties in the refrigerated state, the TBARS values increased with the extension of the storage period. The increase in TBARS values in samples with the addition of 0,5% walnut leaf powder was very slow and remained the lowest (0,15 mg malonaldehyde per kg of sample) up to 15 days of refrigerated storage. Thus, the efficacy of walnut leaf powder in delaying oxidative degradation in prepared pork samples was obvious and comparable to that of BHT at a powder addition level of 0,2%. Basically, it appears that an addition of only 0,2% walnut leaf powder exerts a lipid oxidation delay effect similar to the addition of 0,1% BHT.

The total content of phenolic compounds in pork patties with added walnut powder was significantly (P <0,05) higher compared to control and control with added BHT (Table 6.5.).

Table 6.5. Total content of phenolic compounds in the samples of control patties and containing walnut leaf powder during refrigeration for 15 days (mg GAE/100 g)

	Refrigerated storage period (zile)			
Treatment	0	5	10	15
M0	$7,35 \pm 0,32^{a}$	$6,53 \pm 0,40^{ab}$	$5,68 \pm 0,33^{a}$	$5,42 \pm 0,18^{a}$
M1	$6,85 \pm 0,21^{a}$	$5,87 \pm 0,35^{a}$	$5,17 \pm 0,29^{a}$	$4,95 \pm 0,25^{a}$
P1	$9,00 \pm 0,38^{b}$	$8,18 \pm 0,26^{b}$	$8,00 \pm 0,36^{b}$	$8,57 \pm 0,44^{b}$
P2	$12,27 \pm 0,56^{c}$	$13,07 \pm 0,66^{c}$	$14,22 \pm 0,69^{c}$	$12,68 \pm 0,57^{c}$

* the averages in the same column accompanied by different letters to the exponent differ significantly (P < 0,05)

The addition of BHT as well as that of walnut leaf powder caused a significant increase in antioxidant activity compared to the control (Table 6.6). Also, the increase in the addition of powder from walnut leaves led to a significant increase in free radical scavenging activity..

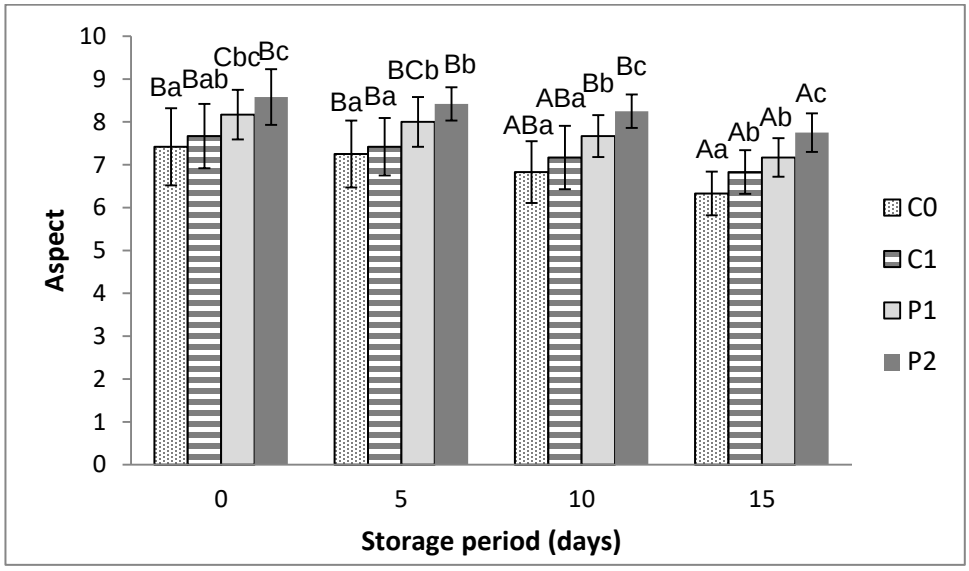
Table 6.6. ABTS antioxidant activity of samples of control patties and containing walnut leaf powder during storage in a refrigerated state for 15 days (mmol Trolox/100 g)

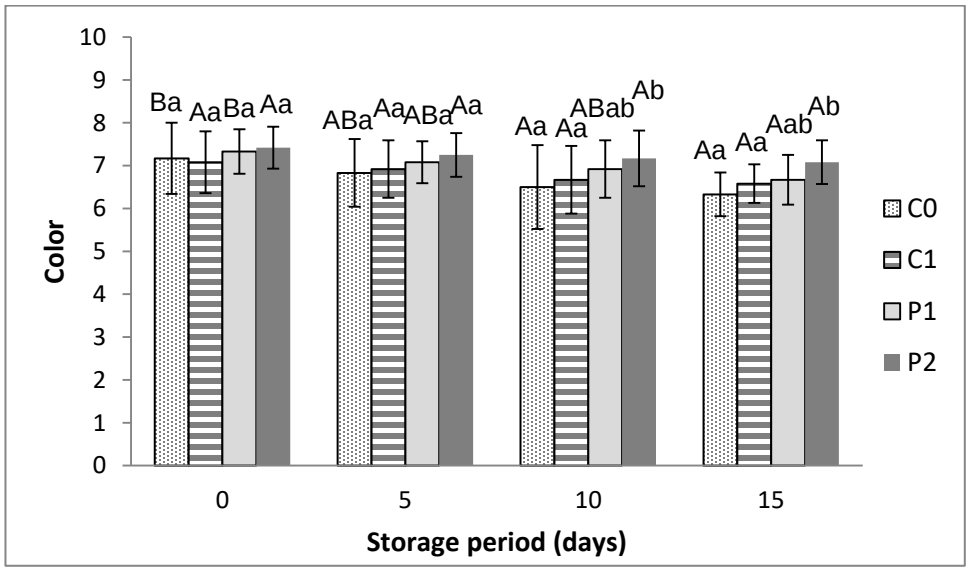
	Refrigerated storage period (days)			
Treatment	0	5	10	15
M0	$0,49 \pm 0,03^{a}$	$0,49 \pm 0,02^{a}$	$0,49 \pm 0,02^{a}$	$0,48 \pm 0,02^{a}$
M1	$0,69 \pm 0,03^{c}$	$0,59 \pm 0,03^{b}$	$0,56 \pm 0,02^{b}$	$0,55 \pm 0,02^{b}$
P1	$0,62 \pm 0,02^{b}$	$0,62 \pm 0,03^{b}$	$0,61 \pm 0,02^{c}$	$0,60 \pm 0,02^{c}$
P2	$0,72 \pm 0,03^{c}$	$0,69 \pm 0,02^{c}$	$0,75 \pm 0,03^{d}$	$0,70 \pm 0,03^{d}$

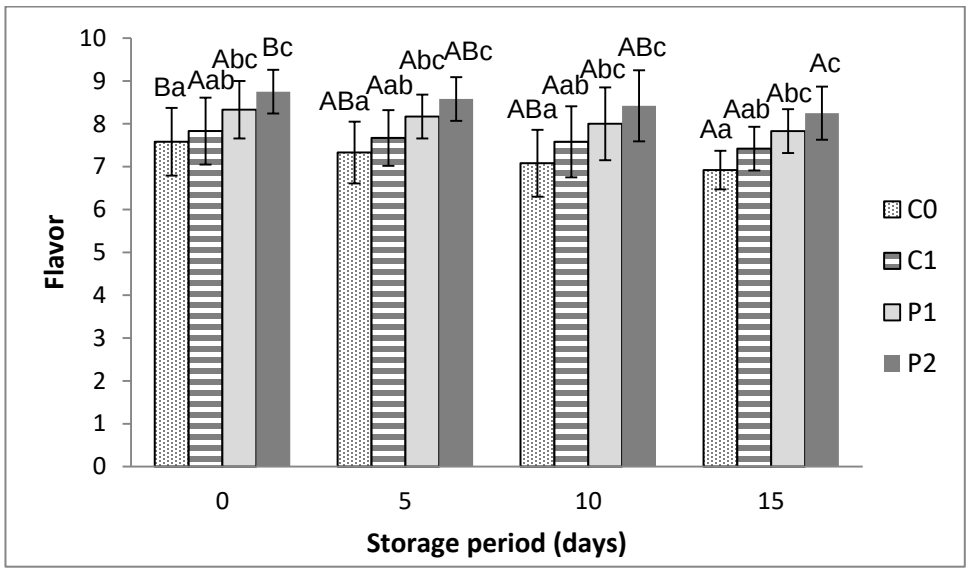
* the averages in the same column accompanied by different letters to the exponent differ significantly (P < 0,05)

Free radical scavenging capacity was not significantly affected during storage, except for BHT-added samples, which recorded a steady decline.

The addition of walnut leaf powder had a positive effect on appearance and aroma during storage (Figure 6.1).


Aspect
Storage period (days)
0
5
10
15
C0
C1
P1
P2


Color
Storage period (days)
0
5
10
15
C0
C1
P1
P2


Flavor
Storage period (days)
0
5
10
15
C0
C1
P1
P2

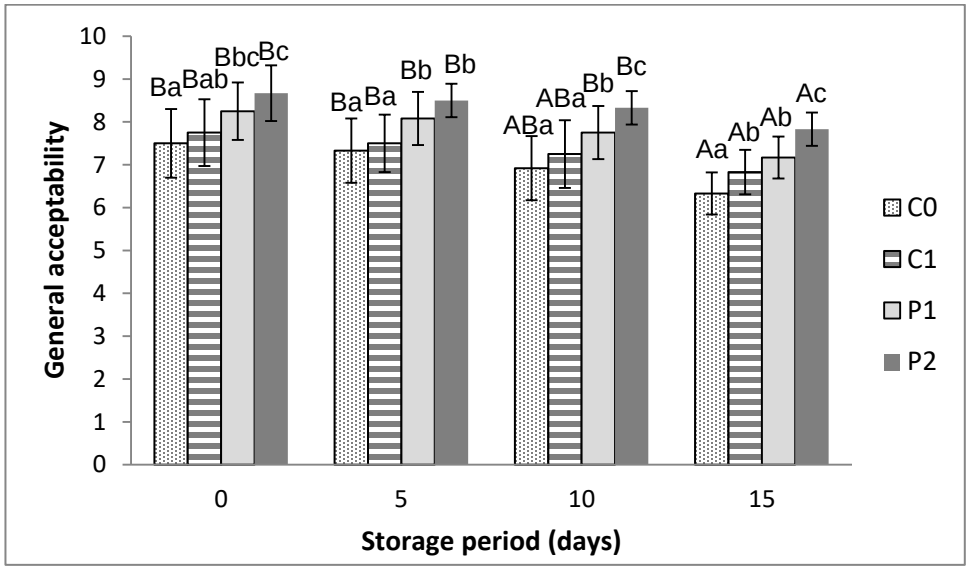


Figure 6.1. Sensory evaluation of samples of control patties and containing walnut leaf powder during storage in a refrigerated state for 15 days. Different lowercase letters indicate significant differences due to treatment (P <0,05), while different uppercase letters indicate significant differences due to retention period (P <0,05).

Significant differences were observed between the beginning and the end of the storage period, the increase in storage time led to a decrease in the overall acceptability of pork patties, with or without additives. However, samples with the addition of walnut leaf powder at 0,5% showed higher scores of overall acceptability and flavor compared to the control, as the powder has the potential to reduce oxidative rancidity and prolong shelf life of cooked pork patties.

6.4. Partial conclusions

Minced pork patties obtained with the addition of walnut leaf powder had lower TBARS values compared to the control during storage, so walnut leaf powder exerted an inhibitory effect on lipid oxidation throughout the period. for storage. Also, the TBARS values in the walnut leaf powder samples increased very slowly during storage for 15 days in the refrigerated state. The results showed that an addition of 0,2% walnut leaf powder exerts a lipid oxidation delay effect similar to the addition of 0,1% BHT.

Walnut leaf powder contributed to the moisture retention in the finished product, which probably contributed to the increase in baking yield. Also, the addition significantly influenced the color, causing the darkening of pork patties (decreasing the parameter L*) and increasing the green component (decreasing the parameter a*). The addition of walnut leaf powder had positive effects on oxidative stability and color in minced cooked pork patties during refrigerated storage for 15 days.

The addition of walnut leaf powder had positive effects on the appearance and aroma both at the initial time and during storage. In conclusion, walnut leaf powder can be a useful ingredient in minced pork, providing a source of natural antioxidants that are safe and improve the oxidative stability of processed meat.

CHAPTER 7

EFFECTS OF WALNUT AND CHERRY STEM EXTRACTS ON COLOR, LIPID OXIDATION AND SENSORY QUALITY OF COOKED PORK PATTIES

7.1. Study Opportunity

The aim of this study was to evaluate the effectiveness of walnut leaf powder and walnut leaf and cherry stem extracts as inhibitors of lipid oxidation and color deterioration of prepared pork patties, subjected to refrigerated storage.

7.2. Materials and methods of analysis

Lean pork meat and back fat were chopped through a 3 mm sieve and mixed to have a 20% back fat ratio. The mixture was divided to provide five experimental variants: I) C0 - Control (meat + 1,5% salt + 5,5% water); II) C1 - BHT control (meat + 1,5% w / w salt + 5,5% water + 0,1% BHT); III) P1 (meat + 1,5% w / w salt + 5,5% walnut leaf extract); IV) P2 (meat + 1,5% w / w salt + 5,5% cherry stem extract) V) P3 (meat + 1,5% w / w salt + 5,5% water + 0,5% walnut leaf powder). After adding all the ingredients, the meat samples were well kneaded and hand-shaped into patties (50 g each). The samples were placed in an electric oven and baked for 10 minutes until the meat reached 75 ± 1°C in the center. After cooling to room temperature, the patties were aerobically packed in polyethylene bags. These were analyzed for sensory characteristics, color, total phenolic compounds content, pH, ABTS antioxidant activity and thiobarbituric acid reagents (TBARS) value at 0, 5, 10 and 15 days of storage at 4°C in darkness.

All determinations were performed in triplicate and the results were reported as mean ± standard deviation. Statistically significant effects were analyzed and averages were compared using the Duncan multiple test. Statistical significance was identified at a 95% confidence level ($P < 0,05$).

7.3. Results and discussion

Chemical composition and baking yield

The moisture content increased significantly ($P < 0,05$) only when walnut leaf powder was added (Table 7.1), indicating that the powder led to moisture retention. These results are consistent with those on baking yield, which was significantly higher in the samples with the addition of walnut leaf powder. The fat and protein contents of the control samples (C0 and C1) were higher than those with additives ($P < 0,05$), probably as a result of the higher moisture loss during the baking of these samples.

The additives had a significant effect on the color of freshly prepared pork patties (day 0) (Table 7.2). The addition of BHT led to patties with significantly higher L* values, the cherry stems extract did not significantly affect the brightness, while the powder from walnut leaves and the extract led to a darker color (lower L*).

Table 7.1. Baking yield, moisture, protein and fat content of pork patties

Treatment	Moisture (%)	Protein (%)	Fat (%)	Baking yeld (%)
C0	$55,39 \pm 0,63^{a}$	$21,68 \pm 0,38^{c}$	$18,62 \pm 0,26^{d}$	$70,8 \pm 1,10^{a}$

C1	55,61 ± 0,40^{a}	21,43 ± 0,46^{bc}	18,39 ± 0,23^{cd}	71,2 ± 1,78^{ab}
P1	55,96 ± 0,52^{ab}	20,86 ± 0,29^{ab}	17,48 ± 0,34^{ab}	72,8 ± 1,10^{b}
P2	55,82 ± 0,29^{a}	21,12 ± 0,33^{abc}	17,88 ± 0,38^{bc}	71,2 ± 1,10^{ab}
P3	56,71 ± 0,44^{b}	20,46 ± 0,36^{a}	17,14 ± 0,28^{a}	72,8 ± 1,10^{b}

* media with different superscript in the column are significantly different (P < 0,05)

Lower L* values may affect the general acceptability of meat products [64]. BHT and plant extracts led to patties with significantly higher a* values, while walnut powder samples had the lowest a* values due to the dark green color of the walnut leaf. Higher b* values (P <0,05) were found in pork patties with BHT added, walnut leaf extract or walnut leaf powder, compared to the control. The red color component (a* value) decreased significantly (P <0,05) during the storage period in all treatments, suggesting that oxidation of the pigment may occur even if antioxidants are added..

Table 7.2. Color parameters of pork patties during refrigerated storage for 15 days

Color parameters	Treatment	Storage period (days)			
		0	5	10	15
L*	C0	71,23 ± 1,49^{bA}	79,53 ± 2,82^{cB}	80,56 ± 3,11^{cB}	78,24 ± 1,32^{dB}
	C1	80,28 ± 0,16^{dB}	78,10 ± 1,27^{cA}	81,09 ± 1,77^{cB}	77,58 ± 1,63^{dA}
	P1	69,77 ± 1,49^{cB}	74,53 ± 1,30^{bB}	72,45 ± 0,61^{bB}	70,06 ± 1,84^{bA}
	P2	71,61 ± 0,71^{bA}	73,70 ± 1,05^{bB}	74,78 ± 2,09^{bB}	73,18 ± 2,22^{cB}
	P3	62,31 ± 2,52^{aA}	65,00 ± 2,24^{aAB}	66,57 ± 0,86^{aB}	62,87 ± 1,20^{aA}
a*	C0	4,47 ± 0,14^{aB}	4,82 ± 0,34^{bB}	3,31 ± 0,27^{aA}	3,15 ± 0,06^{aA}
	C1	7,92 ± 0,42^{dC}	6,24 ± 0,46^{cB}	4,84 ± 0,55^{bA}	4,57 ± 0,26^{bA}
	P1	5,82 ± 0,47^{bA}	6,05 ± 0,33^{cB}	5,36 ± 0,26^{cA}	5,08 ± 0,31^{cA}
	P2	5,93 ± 0,06^{cB}	5,87 ± 0,30^{cB}	5,84 ± 0,26^{cB}	5,10 ± 0,20^{cA}
	P3	4,09 ± 0,38^{aB}	3,68 ± 0,84^{aB}	3,51 ± 0,15^{aAB}	2,86 ± 0,43^{aA}
b*	C0	13,99 ± 0,32^{abAB}	14,06 ± 0,83^{aC}	13,83 ± 0,32^{bA}	14,71 ± 0,37^{bBC}
	C1	15,58 ± 0,43^{dB}	15,11 ± 0,53^{aAB}	14,52 ± 0,80^{cA}	14,67 ± 0,70^{bAB}
	P1	15,35 ± 1,02^{aA}	16,45 ± 0,42^{bD}	14,62 ± 0,44^{cB}	15,39 ± 0,54^{bC}
	P2	13,00 ± 0,51^{cdB}	13,92 ± 0,50^{aA}	13,09 ± 0,09^{aA}	13,13 ± 0,63^{aA}
	P3	14,53 ± 0,97^{bcB}	14,43 ± 1,55^{aB}	13,69 ± 0,25^{abAB}	12,92 ± 0,56^{aA}
C	C0	14,69 ± 0,29^{aA}	15,81 ± 0,89^{aB}	14,22 ± 0,38^{aA}	15,05 ± 0,36^{bAB}
	C1	17,48 ± 0,26^{bB}	16,35 ± 0,62^{abA}	15,31 ± 0,90^{bA}	15,37 ± 0,75^{bcA}
	P1	16,45 ± 1,11^{aA}	17,53 ± 0,50^{bC}	15,57 ± 0,49^{bB}	16,21 ± 0,49^{cB}
	P2	14,29 ± 0,49^{aB}	15,11 ± 0,58^{aA}	14,47 ± 0,45^{aA}	14,08 ± 0,65^{aA}
	P3	15,11 ± 1,02^{aB}	14,90 ± 1,70^{aB}	14,13 ± 0,26^{aAB}	13,23 ± 0,61^{aA}
h	C0	72,27 ± 0,81^{cA}	72,29 ± 0,52^{cA}	76,55 ± 0,81^{dB}	77,92 ± 0,42^{cC}
	C1	63,04 ± 1,76^{aA}	67,57 ± 1,11^{aB}	71,61 ± 1,35^{cC}	72,73 ± 0,19^{bC}
	P1	68,94 ± 0,30^{bA}	69,81 ± 0,57^{bAB}	69,88 ± 0,42^{bB}	71,70 ± 1,37^{bC}
	P2	68,48 ± 0,65^{bC}	67,15 ± 0,32^{aB}	66,20 ± 0,36^{aA}	68,17 ± 0,46^{aC}
	P3	74,27 ± 0,91^{dA}	75,83 ± 1,85^{dAB}	75,63 ± 0,54^{dAB}	77,52 ± 1,56^{cB}

* Different lowercase letters indicate a significant difference (p <0,05) between different treatments, while different capital letters indicate a significant difference (p <0,05) in each treatment between different storage periods.

pH values ranged from 5,79 to 6,29 during 15 days of storage (Table 7.3). At the beginning of the experiment (day 0), the pH values of the samples treated with walnut leaf extract and walnut leaf powder were significantly (P <0,05) lower than in the control samples. This effect could be attributed to the high level of phenolic acids in walnut leaf extract and powder. In all treatments, pH values increased significantly (p <0,05) during 15 days of storage. Walnut leaf powder samples reached higher pH values (P <0,05) than control samples after 15 days of storage, while no significant differences were found between the other samples. This is due to the higher microbial load of walnut leaf powder compared to heat treated extracts.

The total content of phenolic compounds was significantly higher (P <0,05) in treatments P1, P2 and P3 compared to the control samples C0 (7,17 mg / 100 g) and C1 (6,90 mg / 100 g) . Among the treatments, walnut leaf powder significantly increased (P <0,05) the content of phenolic compounds (15,62 mg GAE / 100 g) in pork patties, followed by cherry stem extracts (9,98 mg GAE / 100 g) and walnut leaves (9,72 mg GAE / 100 g). The evolution of the phenolic content indicated a continuous and significant decrease (p <0,05) until the 15th day of storage. The percentage decrease in total phenolic compounds during storage was significantly (P <0,05) higher in C1 (42,7%), followed by C0 (41,7%), P2 (40,6%), P3 (32,6%) and P1 (23,0%) (Table 7.3). These results showed that the treatments with walnut leaf powder and extracts showed significantly higher contents of phenolic compounds (P <0,05) during the storage period.

The overall antioxidant activity of the samples treated with powder and extracts was significantly higher (P <0,05) even compared to the samples with the addition of BHT (Table 7.3). Free radical scavenging activity decreased during storage in all meat samples.

After 15 days of storage, the antioxidant activity was significantly higher in the samples with the addition of extracts rich in phenolic compounds and walnut leaf powder compared to the control samples, while no significant difference was found between the control samples without antioxidant and those with BHT.

Table 7.3 illustrates the effect of treatments and storage on lipid oxidation (TBARS values, measured in mg of malondialdehyde per kg of sample) in cooked pork patties, stored in aerobic conditions for 15 days. The results show that lipid oxidation, measured by TBARS values, was delayed in the samples treated with antioxidants. The initial level of lipid oxidation (day 0) was between 0,88 and 2,56 mg MDA / kg in meat samples. The TBARS values of cooked pork patties prepared with the addition of walnut leaf powder and extracts were significantly lower than those of the other samples at baseline. A stronger antioxidant effect was observed with the addition of walnut leaf extract than with cherry stems, as a lower concentration of malondialdehyde was achieved in pork patties (0,98 vs 1,33, for P1 and respectively P2). The same trend was observed on day 10 of storage, with significant differences between samples (P <0,05).

*Table 7.3. TBARS values, pH, total phenolic compounds content and ABTS antioxidant activity of pork patties during refrigerated storage for 15 days**

	Storage period (days)			
Treatment	0	5	10	15
	TBARS values (mg MDA/kg)			
C0	$2,56 \pm 0,18^{dA}$	$2,74 \pm 0,13^{dAB}$	$2,84 \pm 0,15^{eAB}$	$3,01 \pm 0,26^{dB}$
C1	$1,80 \pm 0,11^{cA}$	$2,08 \pm 0,14^{cB}$	$2,36 \pm 0,09^{dC}$	$2,43 \pm 0,18^{cC}$
P1	$0,98 \pm 0,08^{aA}$	$1,49 \pm 0,09^{bB}$	$1,50 \pm 0,10^{bB}$	$1,58 \pm 0,12^{bB}$

P2	$1,33 \pm 0,12^{bA}$	$1,52 \pm 0,06^{bA}$	$1,78 \pm 0,14^{cB}$	$1,83 \pm 0,15^{bB}$
P3	$0,88 \pm 0,06^{aA}$	$0,93 \pm 0,05^{aA}$	$1,07 \pm 0,08^{aB}$	$1,19 \pm 0,08^{aB}$
pH				
C0	$6,03 \pm 0,02^{bA}$	$6,02 \pm 0,02^{aA}$	$6,07 \pm 0,03^{aB}$	$6,16 \pm 0,02^{aC}$
C1	$5,95 \pm 0,03^{bA}$	$6,05 \pm 0,02^{aAB}$	$6,13 \pm 0,08^{abBC}$	$6,20 \pm 0,09^{abC}$
P1	$5,82 \pm 0,08^{aA}$	$6,02 \pm 0,05^{aB}$	$6,18 \pm 0,05^{bC}$	$6,22 \pm 0,07^{abC}$
P2	$5,97 \pm 0,08^{bA}$	$6,02 \pm 0,03^{aA}$	$6,21 \pm 0,05^{bB}$	$6,24 \pm 0,06^{abB}$
P3	$5,79 \pm 0,09^{aA}$	$6,04 \pm 0,04^{aB}$	$6,20 \pm 0,08^{bC}$	$6,29 \pm 0,07^{bC}$
Total content of phenolic compounds (mg GAE/100 g)				
C0	$7,17 \pm 0,32^{aC}$	$7,02 \pm 0,40^{aBC}$	$6,47 \pm 0,33^{bB}$	$4,18 \pm 0,18^{aA}$
C1	$6,90 \pm 0,21^{aC}$	$6,70 \pm 0,35^{aC}$	$5,20 \pm 0,29^{aB}$	$3,95 \pm 0,25^{aA}$
P1	$9,72 \pm 0,38^{bC}$	$9,42 \pm 0,26^{cBC}$	$8,85 \pm 0,36^{cB}$	$7,48 \pm 0,44^{cA}$
P2	$9,98 \pm 0,56^{bC}$	$8,08 \pm 0,66^{bB}$	$6,22 \pm 0,69^{bA}$	$5,62 \pm 0,57^{bA}$
P3	$15,62 \pm 0,49^{cC}$	$14,87 \pm 0,82^{dBC}$	$14,15 \pm 0,56^{dB}$	$10,53 \pm 0,61^{dA}$
ABTS antioxidant activity (mmol Trolox/100 g)				
C0	$0,55 \pm 0,03^{aB}$	$0,52 \pm 0,02^{aB}$	$0,47 \pm 0,02^{aA}$	$0,45 \pm 0,02^{aA}$
C1	$0,61 \pm 0,03^{bC}$	$0,56 \pm 0,03^{aB}$	$0,49 \pm 0,02^{aA}$	$0,47 \pm 0,02^{aA}$
P1	$0,66 \pm 0,02^{bB}$	$0,63 \pm 0,03^{bAB}$	$0,63 \pm 0,02^{bAB}$	$0,60 \pm 0,02^{bA}$
P2	$0,77 \pm 0,03^{cB}$	$0,76 \pm 0,02^{cAB}$	$0,73 \pm 0,03^{cAB}$	$0,71 \pm 0,03^{cA}$
P3	$0,85 \pm 0,03^{dB}$	$0,84 \pm 0,03^{dB}$	$0,74 \pm 0,03^{cA}$	$0,73 \pm 0,03^{cA}$

* Different lowercase letters indicate a significant difference ($p < 0,05$) between different treatments, while different capital letters indicate a significant difference ($p < 0,05$) in each treatment between different storage periods.

After 15 days of storage, the antioxidant activity was significantly higher in the samples with the addition of extracts rich in phenolic compounds and walnut leaf powder compared to the control samples, while no significant difference was found between the control samples without antioxidant and those with BHT.

On the other hand, storage also affected the MDA concentration, and the TBARS values gradually increased with increasing storage time in all samples, ranging from 1,09 to 3,21 mg MDA / kg after 15 days to 4°C. However, during all storage intervals the TBARS values were higher ($P < 0,05$) in the control samples without antioxidant than those containing walnut leaf extracts and cherry stems. During the 15-day storage period, a 49% and 41% reduction was obtained in roasted pork patties with walnut leaf extract and cherry stems, therefore, the addition of these extracts had significant reduction effects of lipid oxidation in minced pork samples. Walnut leaf extracts were more effective against lipid oxidation than those in cherry stems, which is consistent with the results of in vitro analyzes. Walnut leaf extract had a higher flavonoid content than cherry stem extract, which is directly correlated with the higher antioxidant capacity (3,67 versus 2,84 mmol / 100g Trolox for walnut leaf extract and respectively cherry stems) (Table 5.1) and, consequently, with the more intense antioxidant effect of the first extract.

The data from the sensory evaluation of cooked pork patties are presented in Table 7.4. After ripening (day 0), there were no significant differences in the overall appearance, flavor and general acceptability of the control and treated samples, with the exception of patties treated with walnut leaf powder whose scores were significantly higher.

Table 7.4. Sensory evaluation of pork patties during storage under refrigeration conditions for 15 days

	Treatment	Storage period (days)			
		0	5	10	15
Aspect	C0	$7,25 \pm 0,62^{a}$	$7,08 \pm 0,51^{a}$	$6,75 \pm 0,45^{a}$	$6,25 \pm 0,45^{a}$
	C1	$7,42 \pm 0,51^{a}$	$7,33 \pm 0,49^{ab}$	$7,08 \pm 0,51^{ab}$	$6,75 \pm 0,62^{b}$
	P1	$7,67 \pm 0,49^{a}$	$7,58 \pm 0,51^{b}$	$7,50 \pm 0,52^{c}$	$7,08 \pm 0,29^{b}$
	P2	$7,50 \pm 0,52^{a}$	$7,42 \pm 0,51^{ab}$	$7,33 \pm 0,49^{bc}$	$6,92 \pm 0,29^{b}$
	P3	$8,33 \pm 0,49^{b}$	$8,33 \pm 0,49^{c}$	$8,17 \pm 0,39^{d}$	$7,83 \pm 0,39^{c}$
Color	C0	$6,67 \pm 0,75^{a}$	$6,58 \pm 0,51^{a}$	$6,42 \pm 0,51^{a}$	$6,25 \pm 0,45^{a}$
	C1	$7,08 \pm 0,79^{ab}$	$7,08 \pm 0,79^{ab}$	$6,92 \pm 0,67^{b}$	$6,75 \pm 0,45^{b}$
	P1	$7,33 \pm 0,98^{bc}$	$7,25 \pm 0,87^{bc}$	$7,17 \pm 0,83^{b}$	$7,00 \pm 0,74^{b}$
	P2	$7,25 \pm 0,45^{b}$	$7,08 \pm 0,51^{ab}$	$6,83 \pm 0,39^{ab}$	$6,92 \pm 0,29^{b}$
	P3	$7,83 \pm 0,39^{c}$	$7,75 \pm 0,45^{c}$	$7,67 \pm 0,49^{c}$	$7,75 \pm 0,45^{c}$
Flavor	C0	$7,67 \pm 0,65^{a}$	$7,33 \pm 0,49^{a}$	$7,17 \pm 0,58^{a}$	$6,42 \pm 0,51^{a}$
	C1	$7,75 \pm 0,62^{a}$	$7,42 \pm 0,51^{a}$	$7,33 \pm 0,49^{ab}$	$6,67 \pm 0,49^{ab}$
	P1	$7,67 \pm 0,65^{a}$	$7,58 \pm 0,67^{a}$	$7,42 \pm 0,51^{ab}$	$7,08 \pm 0,79^{b}$
	P2	$7,58 \pm 0,51^{a}$	$7,42 \pm 0,51^{a}$	$7,25 \pm 0,62^{a}$	$7,00 \pm 0,60^{b}$
	P3	$8,33 \pm 0,49^{b}$	$8,17 \pm 0,39^{b}$	$7,75 \pm 0,45^{b}$	$7,58 \pm 0,51^{c}$
General acceptability	C0	$7,42 \pm 0,51^{a}$	$7,08 \pm 0,29^{a}$	$6,92 \pm 0,67^{a}$	$6,00 \pm 0,60^{a}$
	C1	$7,50 \pm 0,52^{a}$	$7,17 \pm 0,58^{a}$	$7,08 \pm 0,51^{ab}$	$6,25 \pm 0,75^{ab}$
	P1	$7,42 \pm 0,51^{a}$	$7,33 \pm 0,49^{a}$	$7,17 \pm 0,58^{ab}$	$6,67 \pm 0,65^{b}$
	P2	$7,33 \pm 0,49^{a}$	$7,17 \pm 0,39^{a}$	$7,00 \pm 0,43^{a}$	$6,58 \pm 0,51^{b}$
	P3	$8,08 \pm 0,29^{b}$	$7,92 \pm 0,51^{b}$	$7,50 \pm 0,52^{b}$	$7,25 \pm 0,45^{c}$

* the averages with different superscript in a column are significantly different (P <0,05)

After 15 days of cold storage, pork patties with extracts of walnut leaves and cherry stems were sensory acceptable, and the addition of extracts had positive effects on sensory attributes. Therefore, these extracts have the potential to reduce the degree of oxidative rancidity and to improve the acceptability and shelf life of cooked pork patties. At the end of the storage period, the scores for the samples treated with walnut leaf powder were significantly higher than those of the control samples and the other treatments. No significant differences were found between the scores of samples treated with extracts rich in phenolic compounds and the samples treated with BHT, while the scores for control samples without antioxidant were significantly lower, especially in terms of color.

7.4. Partial conclusions

From the results obtained it can be concluded that walnut leaf powder and walnut leaf extracts and cherry stems have a high content of phenolic compounds and a high antioxidant activity. The addition of these natural products rich in antioxidant phenolic compounds to pork patties has been shown to be more effective than an addition of 0,1% BHT on slowing lipid oxidation and color deterioration during refrigerated storage. The lowest values of lipid oxidation were found in pork patties with the addition of walnut leaf powder, which showed a strong antioxidant effect. Walnut leaf extract was more effective against lipid oxidation than

cherry stem extract, which is consistent with the results of in vitro tests. The addition of walnut leaf extracts and cherry stems did not affect the appearance, flavor and overall acceptability of cooked pork patties at an addition level of 5,5% tested in this study, while the addition of leaf powder walnut at 0,5% had significant positive effects on sensory attributes. Walnut leaves and cherry stems, as natural plant materials with a high content of phenolic compounds, can be promising sources of natural antioxidants for use in meat products.

CHAPTER 8

ANTIMICROBIAL ACTIVITY OF WALNUT LEAF POWDER AND EXTRACTS OF WALNUT LEAVES AND CHERRY STEMS IN COOKED PORK PATTIES

8.1. Study Opportunity

Despite their well-known pharmaceutical properties and the remarkable antioxidant activity of walnut leaves and cherry stems, there is no information on the inhibitory effects of these plant products on microbial growth in meat products. Therefore, the objectives of these studies were to investigate the potential use of walnut leaf powder and extracts of walnut leaves and cherry stems as natural antimicrobials in cooked meat systems.

8.2. Materials and methods of analysis

For microbiological analysis, 1 g of sample was sterile weighed and homogenized with 9 ml of peptone saline in a stomach. After homogenization, the samples were kept at room temperature for 15 minutes. The seeding was done directly from the sample obtained without performing decimal dilutions. The qualitative determination of the germs without their quantification was followed. The culture media used were XLD agar selective for Enterobacteriaceae as well as nutrient agar as usual medium. Inoculation was done in both cases by grooving on the surface of the environment. Incubation of seed plates was performed at 37℃ for Enterobacteriaceae for 48 hours, and those with nutrient agar were incubated at 30℃ for 48 hours.

The antimicrobial activity of the aqueous extracts added to the patties was tested on the most commonly encountered bacteria in the analyzed samples using the disc diffusion assay. Briefly, the surface of the nutrient agar culture medium was inseminated by flooding the medium with a suspension of Bacillus isolated bacteria from the samples of the analyzed patties. Turbidity values of the suspension was in the range of 2.0–2.2 nephelometric units. Plates with a diameter of 90 mm were used, inoculum being 1 ml of bacterial suspension. After 15 minutes, the excess suspension was removed and 3 sterilized dishes, 6 mm in diameter, impregnated with walnut leaf aqueous extract, sweet cherry stem aqueous extract and distilled water (control) were applied to the surface of the inseminated medium. The placing of the discs on the plate was equidistant. The plates thus obtained were thermostated at 37°C for 48 hours. Plate reading was done by scoring the inhibition area around the inseminated disc, as well as three-way measurement of the inhibition zone (positive samples). The analysis was carried out in duplicate.

The microbiological analysis of the patties samples with the addition of plant extracts and the control sample was performed in two stages: immediately after obtaining and after 15 days of storage in refrigerated conditions..

8.3. Results and discussion

Microbiological analysis of the patties immediately after production

On the agar selective media for Enterobacteraiaceae, no colonies were developed in the thermostatic interval, as a result, the samples were free from pathogenic bacteria of this family. Instead, many colonies have developed on nutrient agar, with varying degrees of uniformity from one sample to another.

In the control samples, after only 24 hours of thermostation on the nutrient agar medium, grayish white colonies with rhizoid margin and slightly gelatinous consistency were observed (figure 1). The degree of uniformity of colonies was very high, indicating that it is a single species of bacteria that has developed in this usual medium.

At the microscope, the Gram-stained smears highlighted sporulated Gram (+) bacilli, with terminal seam, which slightly exceeds the diameter of the vegetative cell. Based on cultural, morphological and biochemical tests, the isolate was classified as Bacillus cereus var. mycoides.

The lowest load of microorganisms was found in the P1i sample. Two types of colonies were developed on the nutrient agar medium, some of them white, with full margin, glossy surface, and creamy consistency.

The microscope showed small, non-sporulated G (+) bacilli belonging to the lactic bacteria (Lactobacillus) naturally present in the meat microflora.

Under the microscope were highlighted G (+) shells characteristically placed in the form of clusters, typical of the genus Staphylococcus.

The P2i sample showed an abundant colonial growth with a high degree of colony uniformity. The colonies developed were creamy, creamy white, with a slightly chalky surface, wavy edges. G (+) bacilli with rounded heads, large, characteristically united in chains, sporulated were observed under the microscope. Based on morphological, cultural and biochemical tests, the colonial isolate was included in the species Bacillus cereus.

The P3i sample showed less intense colonial growth. In this case, two types of colonies developed (figure 1). The predominant translucent colonies with a tendency for white-gray extension were developed. These were generated by unsporulated, small Gram (+) bacilli, typical of the Brochothrix thermosphacta genus that is part of the native microflora of the meat. A small number of colonies were small in size, opaque, white-cream colored. At the microscope, small Gram (+) sporulated bacilli were observed with rounded, spherical ends. This kind of grouping, correlated with the Gram character, cultural aspects and biochemical tests, can placed the isolate in the genus Bacillus spp.

Microbiological analysis of patties after storage for 15 days in refrigerated state

After 15 days of storage in refrigeration conditions, there is a great deal of uniformity between samples, meaning that the colonies have grown abundantly throughout.

Sporulated G (+) bacilli, typical of the genus Bacillus, were observed under the microscope. It should be noted that in the case of the sample with the addition of walnut leaf extract, although no germs belonging to the genus Bacillus were isolated, spores were present that germinated under refrigeration conditions, the resulting vegetative cells multiplying abundantly.

Regarding the susceptibility of bacteria of the genus Bacillus to the action of the aqueous extracts of walnut leaves and cherry stems, it was found that they did not inhibit the studied bacteria, some of them growing even on the impregnated disc. Instead, colonies were found to be weaker.

8.4. Partial conclusions

The addition of aqueous extracts of walnut leaves or cherry stems and walnut leaf powder to the cooked pork patties had no bacteriostatic effect on the local microflora. In the control samples, abundant germs of the genus Bacillus have developed while in the samples with addition of walnut leaf extract, these were inhibited by the added extract, the lactic bacteria present in the native microflora have developed and Staphylococcus have developed very poorly.

In the samples with addition of cherry stem extract, there was a proliferation of germs of the genus Bacillus, while in the samples with addition of walnut leaf powder there were developed germs present in the native microflora such as Brochothrix thermosphacta, but also Bacillus spp.

Plant extracts added to the pork patties had no effect on bacteria of the genus Bacillus, which inhibited the rest of the native microflora, thus becoming dominant, so that it developed abundantly in all analyzed samples after 15 days of storage under refrigeration conditions..

CHAPTER 9

EFFICACY OF WALNUT LEAVES AND SWEET CHERRY STEMS AS NATURAL ANTIOXIDANTS IN RAW PORK PATTIES DURING FROZEN STORAGE

9.1. Study Opportunity

During the processing and storage of meat, lipid oxidation causes changes in color, flavor and nutritional value and affects meat safety. The objective of this work was to investigate the potential of using infusions from walnut leaves and sweet cherry stems to enhance the frozen storage oxidative stability of raw pork patties subjected to chilled storage. The color, pH, total phenolic content and antioxidant activity of frozen raw pork patties were also evaluated during nine months of storage at -18 oC. Butylated hydroxytoluene (BHT) was used as a positive control in this study.

9.2. Materials and methods of analysis

Fresh lean pork meat and back fat were purchased from a local market. They were clean, cut into cubes, ground through a 3 mm plate and mixed to contain 21% back fat. The mixture was divided into four treatments as follows: I) C0 - Control (meat batter + 1,5% salt + 5,5% water); II) C1 - Control with BHT (meat batter + 1,5% w/w salt + 5,5% water + 0,1% BHT); III) WLE (meat batter + 1,5% w/w salt + 5,5% walnut leaf extract); IV) CSE (meat batter + 1,5% w/w salt + 5,5% cherry stem extract). Salt and BHT were dissolved in water or plant extract prior to mixing with the meat batter. Immediately after adding all ingredients, meat samples were thoroughly mixed and made into patties manually (50 g each). The patties were aerobically packed in polyethylene bags and analyzed within 24 h, and after 3, 6, and 9 months of storage at -18°C. For the analysis, the samples were thawed for 12 hours at 6 ± 2°C.

9.3. Results and discussion

Initially, significant differences in pH ($P<0,05$) were found among treatments. The raw patties with BHT and those with walnut leaf extract had lower pH value compared with the control patties. Over nine months of frozen storage, the pH values of the raw pork patties significantly increased ($P<0,05$) in all samples, probably as a result of the exposure of basic groups by protein denaturation. There were no significant differences ($P<0,05$) in the pH values between treatments in raw pork patties after nine months of storage in the frozen state.

The lipid oxidation products were measured by means of TBARS values, expressed as mg malonaldehyde per kilogram of meat sample (table 9.1). Immediately after preparation, the oxidation of lipids in raw patties was relatively low in all samples, however the TBARS values of raw pork patties treated with walnut leaf extracts (EFN) and cherry stem extracts (ECC) were were significantly lower ($P <0,05$) than that of the control without antioxidants (C0) and samples with the addition of BHT (C1).

A considerable increase in TBARS values was observed in all treatments during the frozen storage period (-18°C). However, throughout the storage period, TBARS values were significantly lower ($P <0,05$) in patties with the addition of natural extracts than in control

samples, suggesting that extracts from walnut leaves and cherry stems were effective in protecting raw meat patties from lipid oxidation. After nine months of frozen storage, the MDA level in control patties increased to 2,17 mg MDA / kg, while in the samples treated with walnut leaf extracts and cherry stems they were only 0,72 and respectively 1,01 mg MDA / kg (Table 9.1).

Table 9.1. TBARS values, pH, total phenolic content and ABTS antioxidant activity of the raw pork patties during frozen storage for 9 months

	Storage period (months)			
Treatment	0	3	6	9
TBARS values (mg MDA/kg)				
C0	0.74 ± 0.03^{cA}	0.94 ± 0.03^{dB}	1.24 ± 0.05^{dC}	2.17 ± 0.12^{dD}
C1	0.28 ± 0.02^{bA}	0.45 ± 0.03^{cB}	0.75 ± 0.04^{cC}	1.22 ± 0.07^{cD}
EFN	0.12 ± 0.02^{aA}	0.25 ± 0.02^{aB}	0.43 ± 0.02^{aC}	0.72 ± 0.04^{aD}
ECC	0.16 ± 0.02^{aA}	0.32 ± 0.02^{bB}	0.53 ± 0.03^{bC}	1.01 ± 0.04^{bD}
pH				
C0	5.83 ± 0.04^{cA}	6.04 ± 0.05^{bB}	6.16 ± 0.04^{aC}	6.28 ± 0.05^{aD}
C1	5.70 ± 0.03^{abA}	6.02 ± 0.02^{bB}	6.10 ± 0.05^{aC}	6.24 ± 0.05^{aD}
EFN	5.68 ± 0.05^{aA}	6.01 ± 0.03^{bB}	6.11 ± 0.03^{aC}	6.24 ± 0.04^{aD}
ECC	5.77 ± 0.04^{bcA}	5.92 ± 0.04^{aB}	6.11 ± 0.04^{aC}	6.20 ± 0.04^{aD}
Total phenolic content (mg GAE/100 g)				
C0	8.80 ± 0.30^{aA}	8.48 ± 0.40^{aA}	8.34 ± 0.30^{aA}	8.40 ± 0.50^{aA}
C1	8.65 ± 0.40^{aA}	8.56 ± 0.30^{aA}	8.49 ± 0.40^{aA}	8.36 ± 0.40^{aA}
EFN	17.03 ± 0.60^{cD}	15.92 ± 0.60^{cC}	14.02 ± 0.50^{cB}	12.55 ± 0.50^{cA}
ECC	12.97 ± 0.50^{bD}	12.08 ± 0.50^{bC}	10.58 ± 0.40^{bB}	8.98 ± 0.30^{bA}
ABTS antioxidant activity (mmol Trolox/100 g)				
C0	0.47 ± 0.02^{aD}	0.41 ± 0.01^{aC}	0.33 ± 0.02^{aB}	0.26 ± 0.01^{aA}
C1	0.60 ± 0.02^{bD}	0.53 ± 0.02^{bC}	0.47 ± 0.02^{bB}	0.38 ± 0.02^{bA}
EFN	0.76 ± 0.03^{dD}	0.70 ± 0.03^{dC}	0.60 ± 0.02^{cB}	0.52 ± 0.02^{dA}
ECC	0.71 ± 0.02^{cD}	0.64 ± 0.02^{cC}	0.56 ± 0.03^{cB}	0.45 ± 0.02^{cA}

* Different lowercase letters indicate significant difference at p<0,05 level between different treatments, while different uppercase letters are indicative of the same within each treatment during the storage period.

Walnut leaf extract significantly increased (P <0,05) the content of phenolic compounds in raw pork patties, followed by cherry stems extract. Changes in the content of phenolic compounds during storage (Table 9.1) indicated a significant decrease (P <0,05) in them until the 9th month of frozen storage in patties with polyphenol-rich extracts. At the end of the storage period, patties with walnut leaf extract had the highest content of phenolic compounds (12,55 mg GAE/100 g).

According to the ABTS analysis, the addition of plant extracts resulted in a significant increase (P <0,05) in antioxidant activity compared to control samples and the addition of BHT (Table 9.1). Free radical scavenging activity decreased significantly during the 9-month storage period. The antioxidant activity was significantly (P <0,05) higher for the treatment with walnut leaf extract compared to the treatment with cherry stem extract, both at the beginning and at the end of the storage period.

The evolution of the color parameters L*, a* and b* is presented in Table 9.2. The brightness (remains L*) a significantly higher (P <0,05) higher in control and control with the addition of BHT compared to the samples with the addition of plant extract (EFN and ECC). The a* values of the patties treated with walnut leaf extracts were higher than those of the control on day 0 (immediately after the patties were prepared), but no significant differences (P <0,05) were found between them at the end of the period. for storage.

All types of patties experienced a significant decrease in a* values during freezing storage, indicating a change in color from red to brown, which could be due to the formation of metmioglobin due to oxidation of the pigment myoglobin. [5].

*Table 9.2. Color parameters of raw pork patties during storage for 9 months in the frozen state **

Color parameters	Treatment	Storage period (months)			
		0	3	6	9
L*	C0	74.16 ± 0.31^{cD}	70.65 ± 0.32^{cC}	67.14 ± 0.71^{bB}	65.38 ± 0.93^{bA}
	C1	72.60 ± 0.91^{bC}	70.43 ± 0.45^{cB}	68.27 ± 1.07^{bA}	67.19 ± 1.49^{bA}
	EFN	68.03 ± 0.86^{aC}	65.92 ± 0.83^{aBC}	63.81 ± 1.89^{aAB}	62.76 ± 2.48^{aA}
	ECC	68.47 ± 0.85^{aA}	68.17 ± 0.58^{bA}	67.88 ± 0.55^{bA}	67.73 ± 0.64^{bA}
a*	C0	10.72 ± 0.57^{aD}	8.51 ± 0.47^{abC}	6.30 ± 0.42^{abB}	5.19 ± 0.41^{aA}
	C1	11.65 ± 0.47^{bD}	9.55 ± 0.49^{cC}	7.45 ± 0.63^{bB}	6.40 ± 0.72^{bA}
	EFN	11.86 ± 0.48^{bC}	9.34 ± 0.90^{bcB}	6.83 ± 1.42^{abA}	5.58 ± 1.70^{aA}
	ECC	10.08 ± 0.79^{aD}	7.87 ± 0.36^{aC}	5.65 ± 0.33^{aB}	5.54 ± 0.53^{aA}
b*	C0	15.19 ± 0.89^{aC}	13.59 ± 0.72^{aB}	11.99 ± 1.39^{aA}	11.19 ± 0.69^{aA}
	C1	14.51 ± 0.75^{aA}	13.95 ± 0.82^{aA}	13.40 ± 1.09^{aA}	13.12 ± 1.26^{aA}
	EFN	15.09 ± 0.62^{aB}	13.89 ± 1.34^{aAB}	12.69 ± 2.09^{aAB}	12.09 ± 2.46^{aA}
	ECC	17.94 ± 1.22^{bC}	16.93 ± 0.53^{bBC}	15.91 ± 0.22^{bAB}	15.41 ± 0.54^{bA}
C	C0	18.59 ± 1.04^{aC}	16.03 ± 0.81^{aB}	13.55 ± 1.30^{aA}	12.34 ± 0.71^{aA}
	C1	18.61 ± 0.86^{aC}	16.91 ± 0.95^{aBC}	15.33 ± 1.24^{abAB}	14.60 ± 1.43^{abA}
	EFN	19.19 ± 0.76^{abC}	16.74 ± 1.61^{aBC}	14.42 ± 2.51^{aAB}	13.33 ± 2.93^{aA}
	ECC	20.58 ± 1.35^{bC}	18.67 ± 0.58^{bB}	16.89 ± 0.32^{bA}	16.06 ± 0.66^{bA}
h	C0	54.78 ± 0.64^{bA}	57.95 ± 1.02^{bB}	62.13 ± 1.52^{aC}	65.09 ± 1.72^{aD}
	C1	51.24 ± 0.68^{aA}	55.60 ± 0.28^{aB}	60.92 ± 0.62^{aC}	64.01 ± 1.01^{aD}
	EFN	51.84 ± 0.58^{aA}	56.07 ± 0.43^{aB}	61.87 ± 1.32^{aC}	65.71 ± 2.85^{aD}
	ECC	60.68 ± 1.49^{cA}	65.08 ± 0.89^{cB}	70.47 ± 0.80^{bC}	73.61 ± 1.31^{bD}

* Different lowercase letters indicate significant difference at p<0,05 level between different treatments, while different uppercase letters are indicative of the same within each treatment during the storage period.

Walnut leaf and cherry stem extracts reduced the decrease of the parameter a* during the frozen storage of pork patties. patties with the addition of cherry stem extract showed significantly higher values of parameter b* (P <0,05) than those of the control, while no significant differences were found between the other treatments. Mean hue values were significantly (P <0,05) higher in samples with cherry stem extract, followed by control and lower in samples treated with BHT and walnut leaf extracts, including there were no significant differences (P <0,05). Hue values increased during storage with all treatments. The values of color intensity (chroma) were significantly higher in the samples with cherry stems extract than in other treatments. Color intensity values were significantly different between BHT, EFN, and

control treatments. Chroma showed a decreasing trend with increasing frozen storage time for all treatments.

9.3. Partial conclusions

The results indicate the potential usage of walnut leaf and cherry stem extracts as efficient inhibitors of lipid oxidation and color deterioration during chilled storage of raw pork patties. Addition of walnut leaf and cherry stem extracts into the raw pork patties limited the oxidative reactions more than BHT, a synthetic antioxidant. These effects could be atributted to the presence of antioxidant phenolic compounds in the extracts, which act as efficient radical scavengers and metal chelators in vitro and retard methmyoglobin formation in patties.

According to the results, the walnut leaf extract was more effective than cherry stem extract in preventing lipid oxidation and inhibiting the browning and discoloration of frozen pork patties by reducing the loss of redness and the increase of yellowness. Using these extracts as natural antioxidants could be an effective strategy to prolong the shelf life of frozen meat products

CHAPTER 10

EFFECT OF CHITOSAN BASED EDIBLE COATING ENRICHED WITH EXTRACTS FROM WALNUT LEAVES AND SWEET CHERRY STEMS ON THE QUALITY OF PRECOOKED PORK PATTIES

10.1. Study Opportunity

This study was performed to evaluate the effectiveness of chitosan-based edible coatings containing walnut leaf extracts and cherry stems extracts against moisture loss and lipid oxidation in precooked pork patties during cold storage.

10.2. Materials and methods of analysis

Pork patties were made according to the recipe: 73,5% lean pork, 20% back fat, 5% ice and 1,5% salt. The meat and fat were chopped through a 3 mm sieve, then ice and salt were added. The mixture was homogenized manually for 10 minutes and formed (manually) into 50 g patties. The samples were cooked in an electric oven until the internal temperature reached 75 ± 1°C. After cooling, the patties were divided into four groups: uncovered (C); coated with chitosan (CH); coated with chitosan with walnut leaf extract (CWL) and coated with chitosan with cherry stems extract (CCS).

Chitosan solution was prepared by dissolving 3 g chitosan in 100 mL of 1% acetic acid aqueous solution with 1 g glycerine. The mixture was heated to boiling (about 100 oC) on a magnetic stirrer/hot plate until the solution became clear, agitated in an ultrasonic bath for 60 min to eliminate bubbles and then kept at room temperature until use for coating. Active edible coatings were made in the same way as above but using infusions of sweet cherry stems and walnut leaves, respectively, as the solvent of the 1% acetic acid solution.

The patties were individually dipped in the coating solutions for 10 s at room temperature and allowed to drain (to remove coating excess) for 10 s. This dipping procedure was repeated three times, then the patties were dried for 2 h in a laminar flow hood. Patties, with or without coating, were individually packed into small polyethylene bags and stored at 2℃. Relative weight loss, pH, instrumental colour, lipid oxidation and antioxidant activity were determined in pork patties at the end of the coating application process and after 5, 10, and 15 days of storage. Lipid oxidation was evaluated by measuring thiobarbituric acid reactive substances (TBARS) while antioxidant activity was measured using the ABTS method. All the assays were performed in triplicate.

10.3. Results and discussion

Weight loss increased significantly ($P < 0,05$) for all samples during the 15-day storage period (Table 10.1). Edible coating significantly reduced the relative weight loss in pork during refrigerated storage. Coating with chitosan resulted in a reduction in relative weight loss by 44,9%, 29,3% and 20,5% compared to the control sample, after 5, 10 and 15 days of cold storage, respectively. Enrichment of chitosan coating with plant extracts did not significantly affect the relative moisture loss of meat samples.

Tabelul 10.1. Relative weight loss (%) of the pork patties during refrigerated storage for 15 days

	Storage period (days)		
Treatment	5	10	15
C	1.67 ± 0.28^{bA}	3.44 ± 0.25^{bB}	4.63 ± 0.22^{bC}
CH	0.92 ± 0.10^{aA}	2.43 ± 0.18^{aB}	3.68 ± 0.32^{aC}
CWL	0.98 ± 0.13^{aA}	2.51 ± 0.26^{aB}	3.80 ± 0.22^{aC}
CCS	1.11 ± 0.14^{aA}	2.63 ± 0.19^{aB}	3.92 ± 0.24^{aC}

* Data represent mean ± standard deviation of three replicates. Different lowercase letters indicate significant differences due to treatment (p<0,05), while different uppercase letters are indicative of significant differences due to storage period (p<0,05).

Edible coatings reduced lipid oxidation of meat samples compared to control (Table 10.2). Coatings with plant extracts were the most effective (44,53% and 37,91% reduction in TBARS compared to the control for CWL and CCS, respectively) and also showed the highest antioxidant activity..

After 15 days of cold storage, the TBARS values of the chitosan-coated samples were 12,7% lower than those of the control samples. This may be due to the antioxidant properties of chitosan and its low oxygen permeability [65]. In addition, oxidation decreased as the coating prevented the interaction between the air and the surface of the meat.

Table 10.2. TBARS values (mg MDA/kg) of the pork patties during refrigerated storage for 15 days

	Storage period (days)			
Treatment	0	5	10	15
C	0.72 ± 0.03^{aA}	1.42 ± 0.09^{bB}	2.39 ± 0.14^{cC}	3.93 ± 0.20^{cD}
CH	0.96 ± 0.05^{cA}	1.30 ± 0.08^{bB}	2.12 ± 0.11^{bC}	3.43 ± 0.16^{bD}
CWL	0.82 ± 0.03^{bA}	1.10 ± 0.06^{aB}	1.55 ± 0.08^{aC}	2.18 ± 0.09^{aD}
CCS	0.78 ± 0.04^{abA}	1.02 ± 0.04^{aB}	1.48 ± 0.07^{aC}	2.44 ± 0.12^{aD}

* Data represent mean ± standard deviation of three replicates. Different lowercase letters indicate significant differences due to treatment (p<0,05), while different uppercase letters are indicative of significant differences due to storage period (p<0,05).

The composition of the chitosan film could also contribute to its lower permeability to O_2. Chitosan is insoluble in water, but is slightly soluble in dilute organic acids [66]. In this study, acetic acid was used as the solvent to form the chitosan coating. In previous studies, acetic acid chitosan film was reported to have a lower O_2 permeability coefficient than lactic acid or formic acid chitosan films [67] but lower water resistance than other chitosan films made with acetic acid. other acids [68]. Compared to the chitosan-coated samples, the TBARS values of CWL and CCS were lower due to the presence of antioxidant extracts, and CWL had the best effect.

Lipid oxidation intensified significantly (P <0,05) during storage, especially in control patties, which showed the highest increase in TBARS values. This can be attributed to the partial dehydration of pork patties and the increased oxidation of unsaturated fatty acids. According to Campo [69], a TBARS value of 2 mg MDA / kg was considered as a threshold for the sensory detection of rancid flavors in beef. In this study, the baseline TBARS value of cooked patties was 0,72-0,96 mg MDA / kg and exceeded 2 mg MDA / kg on day 10 of storage for control and chitosan-coated samples. However, TBARS values for CWL and CCS exceeded 2 mg MDA / kg on day 15.

Chitosan-coated meat samples showed higher antioxidant activity (P <0,05) compared to control samples, probably due to the antioxidant activity of chitosan (Table 10.3). Other studies have shown that chitosan, as a coating, interacts with food surface components, increasing their antioxidant properties [70].

Free radical scavenging activity decreased significantly (P <0,05) during the 15-day storage period. No significant difference was found between CWL and CCS in terms of antioxidant activity of pork patties.

Samples treated with CWL and CCS had a lower pH value than chitosan-coated and control samples (Table 10.4). However, significant differences were found between chitosan-covered patties and other treatments only after 5 days of cold storage. The pH increased at the beginning of the storage period and then decreased slightly.

Table 10.3. ABTS antioxidant activity (μM Trolox/100 g) of the pork patties during refrigerated storage for 15 days

	Storage period (days)			
Treatment	0	5	10	15
C	0.44 ± 0.02^{aD}	0.40 ± 0.02^{aC}	0.36 ± 0.02^{aB}	0.29 ± 0.02^{aA}
CH	0.51 ± 0.03^{bC}	0.46 ± 0.02^{bBC}	0.42 ± 0.03^{bAB}	0.38 ± 0.03^{bA}
CWL	0.75 ± 0.04^{cB}	0.73 ± 0.03^{cB}	0.63 ± 0.03^{cA}	0.61 ± 0.03^{cA}
CCS	0.72 ± 0.03^{cC}	0.68 ± 0.03^{dBC}	0.64 ± 0.02^{cB}	0.58 ± 0.03^{cA}

* Data represent mean ± standard deviation of three replicates. Different lowercase letters indicate significant differences due to treatment (p<0,05), while different uppercase letters are indicative of significant differences due to storage period (p<0,05).

Table 10.4. pH of the pork patties during refrigerated storage for 15 days

	Storage period (days)			
Treatment	0	5	10	15
C	6.07 ± 0.03^{B}	6.22 ± 0.03^{abC}	5.97 ± 0.06^{A}	5.89 ± 0.05^{A}
CH	6.05 ± 0.03^{B}	6.27 ± 0.02^{bC}	5.93 ± 0.03^{A}	5.87 ± 0.05^{A}
CWL	6.02 ± 0.07^{B}	6.19 ± 0.05^{aC}	5.91 ± 0.03^{A}	5.88 ± 0.07^{A}
CCS	6.00 ± 0.06^{B}	6.17 ± 0.02^{aC}	5.92 ± 0.05^{AB}	5.91 ± 0.04^{A}

* Data represent mean ± standard deviation of three replicates. Different lowercase letters indicate significant differences due to treatment (p<0,05), while different uppercase letters are indicative of significant differences due to storage period (p<0,05).

Brightness values increased with storage time for all treatments. The a* values of patties treated with CWL and CCS were lower than those of control samples and those coated with chitosan (CH) on day 0, and this trend was maintained throughout the storage period. There was a significant decrease in redness (a* values) in all samples, indicating a change in color from red to brown.

Table 10.5. Color parameters of the pork patties during refrigerated storage for 15 days

Color parameters	Treatment[1]	Storage period (days)			
		0	5	10	15
L^*	C	60.95 ± 0.38^{aA}	63.20 ± 1.17^{abB}	66.23 ± 1.56^{bC}	67.20 ± 0.93^{cC}
	CH	64.31 ± 0.91^{bA}	64.73 ± 1.57^{bcA}	65.93 ± 2.49^{bAB}	67.64 ± 1.51^{cB}
	CWL	59.93 ± 2.00^{aA}	62.63 ± 0.16^{aB}	62.57 ± 0.54^{aB}	62.59 ± 0.57^{aB}

	CCS	61.98 ± 1.69^{aA}	65.91 ± 0.94^{cB}	69.98 ± 0.56^{cC}	65.41 ± 0.52^{bB}
a*	C	4.34 ± 0.32^{abC}	3.49 ± 0.23^{aB}	3.22 ± 0.07^{aAB}	3.12 ± 0.03^{bA}
	CH	4.56 ± 0.27^{bD}	3.93 ± 0.08^{bC}	3.53 ± 0.17^{abB}	3.08 ± 0.13^{abA}
	CWL	3.85 ± 0.42^{aB}	3.74 ± 0.18^{abB}	3.71 ± 0.84^{abB}	2.76 ± 0.41^{aA}
	CCS	3.87 ± 0.25^{aB}	3.72 ± 0.27^{abB}	3.95 ± 0.12^{bB}	2.77 ± 0.42^{abA}
b*	C	14.91 ± 0.78^{b}	14.75 ± 0.61^{ab}	15.31 ± 0.56^{b}	15.55 ± 0.29^{b}
	CH	15.00 ± 0.85^{b}	15.58 ± 0.34^{c}	15.43 ± 0.76^{b}	15.15 ± 0.54^{b}
	CWL	13.80 ± 0.66^{aA}	14.39 ± 0.34^{aAB}	14.25 ± 0.39^{aAB}	14.85 ± 0.65^{abB}
	CCS	13.75 ± 0.55^{aA}	15.15 ± 0.50^{bcB}	16.76 ± 0.22^{cC}	13.80 ± 0.66^{aA}
C	C	15.53 ± 0.83^{b}	15.16 ± 0.65^{ab}	15.64 ± 0.56^{b}	15.86 ± 0.28^{b}
	CH	15.68 ± 0.89^{b}	16.07 ± 0.34^{c}	15.83 ± 0.78^{b}	15.46 ± 0.55^{b}
	CWL	14.33 ± 0.73^{a}	14.87 ± 0.29^{a}	14.74 ± 0.25^{a}	15.12 ± 0.75^{ab}
	CCS	14.29 ± 0.60^{aA}	15.60 ± 0.54^{bcB}	17.22 ± 0.24^{cC}	14.33 ± 0.73^{aA}
H	C	73.79 ± 0.50^{abA}	76.72 ± 0.37^{bB}	78.11 ± 0.32^{bC}	78.67 ± 0.27^{bC}
	CH	73.09 ± 0.09^{aA}	75.84 ± 0.35^{abB}	77.11 ± 0.34^{abC}	78.53 ± 0.21^{bD}
	CWL	74.45 ± 1.05^{bA}	75.43 ± 0.94^{aA}	75.41 ± 3.46^{aA}	79.54 ± 1.03^{aB}
	CCS	74.29 ± 0.42^{bA}	76.20 ± 0.66^{abB}	76.75 ± 0.23^{abB}	79.07 ± 1.05^{aC}

* Data represent mean ± standard deviation of three replicates. Different lowercase letters indicate significant differences due to treatment ($p<0,05$), while different uppercase letters are indicative of significant differences due to storage period ($p<0,05$)

10.4. Partial conclusions

Results of this study showed that chitosan-based coatings were effective in controlling lipid oxidation in pork patties. The coatings retarded water loss and maintained acceptable values of pH throughout the period studied. The incorporation of walnut leaf and cherry stem extracts in chitosan coatings improved the antioxidant protection, offering an advantage in the prevention of lipid oxidation in meat products. The meat samples with chitosan-based coatings incorporating antioxidant extracts showed lower TBARS values, which remained below the limits of acceptability for 10 days of refrigerated storage. However, further improvements are necessary to develop a more successful application of edible coatings enriched with plant extracts.

CHAPTER 11

FINAL CONCLUSIONS

→ Walnut leaves and cherry stems are a valuable source of phenolic compounds, with immense nutraceutical value, for the development of functional meat products of commercial interest..

→ The use of powders and extracts from walnut leaves and cherry stems as an ingredient in the production of minced pork products improves the nutritional value and storage stability of the product. The addition of these extracts or powders delayed the oxidative degradation of pre-cooked pork patties during refrigerated storage for 15 days, leading to increased antioxidant activity in the product, while having positive effects on sensory properties and overall acceptability of their.

→ An addition of 0,2% walnut leaf powder exerts a lipid oxidation delay effect similar to the addition of 0,1% BHT. In addition, walnut leaf powder helps to retain moisture in the finished product and increase ripening efficiency.

→ Powders and extracts of walnut leaves and cherry stems have the potential as natural and cheap sources of antioxidants for meat and meat products. Moreover, their application in the meat industry can be very valuable and desirable in terms of health benefits given the very high content of highly effective natural antioxidants. The use of walnut leaves and cherry stems to extend the shelf life of pork products could meet the demands of modern consumers for natural, safe and healthy food ingredients..

→ The addition of aqueous extracts of walnut leaves and cherry stems or walnut leaf powder had no bacteriostatic effect on the native microflora of pre-cooked pork patties.

→ Extracts from walnut leaves and cherry stems have been shown to be effective inhibitors of lipid oxidation and color deterioration (browning) during frozen, minced and salted storage of raw pork. The addition of extracts from walnut leaves and cherry stems to pork patties limited oxidative reactions more than BHT, a synthetic antioxidant. These effects could be attributed to the presence of antioxidant phenolic compounds in the extracts, which act as free radical scavengers and delay the formation of metmioglobin in the meat product..

→ Walnut leaf extracts and cherry stems extracts added to chitosan-based coatings improved the antioxidant protection offered by chitosan and had positive effects on the quality of pork patties during refrigeration. Active chitosan coatings delayed moisture loss and maintained acceptable pH values throughout the storage period.

→ Chitosan coating with antioxidant extracts has the potential to meet consumer demand for food without chemical preservatives and can be a good active packaging for extending the shelf life of meat and pork products. Therefore, these coatings may have the potential to be developed into functional food packaging materials and represent a promising alternative to synthetic materials. The use of this type of coating can be considered an emerging technology aimed at extending the lifespan of ready-to-eat meat products.

CHAPTER 12

CONTRIBUTIONS AND PROSPECTS FOR FURTHER RESEARCH

Based on the original experimental results obtained in the thesis, the following can be highlighted as scientific contributions:

→ Obtaining aqueous powders and extracts of walnut leaves and cherry stems and characterizing them in terms of the total content of phenolic compounds, flavonoids, the antioxidant activity of DPPH and ABTS and the phenolic profile.

→ Analysis of the seasonal variation of the total content of phenolic compounds in walnut leaves in order to establish the optimal time of their collection to obtain biologically active powders and extracts.

→ Assessment of oxidative stability and color stability of pre-cooked minced pork meat with the addition of walnut leaf powder at addition levels of 0,2% and 0,5%, compared to a control without antioxidant addition and an active control with additions of 0,1% butylhydroxytoluene.

→ Evaluation of the efficacy of walnut leaf and cherry stem extracts and walnut leaf powder as inhibitors of lipid oxidation and color deterioration of prepared pork patties subjected to refrigerated storage. In addition, these experiments looked at the chemical composition and baking yield, the evolution of pH, the total content of phenolic compounds, antioxidant activity and the evolution of sensory characteristics (appearance, color, taste) and the general acceptability of pre-cooked pork patties.

→ Evaluation of the antimicrobial activity of walnut leaf powder and walnut leaf extracts and cherry stems in cooked pork patties.

→ Investigation of the potential use of infusions of walnut leaves and cherry stems to increase oxidative stability during frozen storage of raw pork patties. The color, pH and antioxidant activity of frozen raw pork paties were also evaluated for nine months of storage at -18°C. Butylhydroxytoluene (BHT) was used as a positive control antioxidant in this study.

→ Evaluation of the efficacy of edible chitosan-based coatings containing walnut leaf extracts and cherry stem extracts to reduce moisture loss and lipid oxidation in precooked pork patties during cold storage.

The results obtained constitute a scientific database that can be the starting point for further research on improving the functional value of meat products by adding powders and natural extracts with antioxidant properties. The food industry can use these plant resources as a source of natural antioxidants in processed foods. Also, active edible coatings that incorporate natural antioxidant extracts can be developed into functional food packaging materials and represent a promising alternative to synthetic materials.

Future research should include studies to optimize procedures for the extraction of biologically active compounds from these plant resources with high antioxidant potential, studies related to the food safety of these products and studies to define optimal dietary combinations and / or minimum levels of powders or extracts. necessary to obtain the greatest stability in the finished product. In addition, other studies should investigate the application of new mixtures of natural antioxidants to develop products with new structures, using techniques such as encapsulation or nanotechnology.

CHAPTER 13

DISSEMINATION OF RESEARCH RESULTS CARRIED OUT ON THE TOPIC OF THE DOCTORAL THESIS

Articles / studies published in ISI listed journals

1. Boruzi A.I., Nour V. 2019. Walnut (Juglans regia L.) leaf powder as a natural antioxidant in cooked pork patties. CyTA - Journal of Food, 17(1), 431-438, DOI: 10.1080/19476337.2019.1596984 (IF=1,605)
https://www.tandfonline.com/doi/full/10.1080/19476337.2019.1596984

2. Boruzi A.I., Nour V. 2019. Antioxidant effects of walnut leaves and sweet cherry stems on color, lipid oxidation and sensory quality of cooked pork patties. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 47(3), 47(3), 763-771, DOI:10.15835/nbha47311458 (IF=0,648)
https://www.notulaebotanicae.ro/index.php/nbha/article/view/11458

3. Boruzi A.I., Nour V. 2020. Effect of chitosan based edible coating enriched with extracts from walnut leaves and sweet cherry stems on the quality of precooked pork patties. Journal of Food Safety and Food Quality – accept de publicare

Articles / studies published in ISI emerging journals

1. Boruzi A.I., Nour V. 2019. Efficacy of walnut leaves and sweet cherry stems as natural antioxidants in raw pork patties during frozen storage – Scientific Study & Research - Chemistry & Chemical Engineering, Biotechnology, Food Industry, 20 (4), pp. 551 – 561. ISSN 1582-540X. http://pubs.ub.ro/?pg=revues&rev=cscc6&num=201904&vol=4&aid=4955

Articles / studies published in journals indexed in international BDI databases

1. Boruzi A.I., Nour V. 2016. Reduction or replacement of nitrite in processed meat products. Analele Universităţii din Craiova, seria Biologie, Horticultură, Tehnologia Prelucrării Produselor Agricole, Ingineria Mediului, vol. XXI (LVII), Craiova, 277-282. ISSN 1435-1275.http://horticultura.ucv.ro/horticultura/analele-universitatii-din-craiova-seria-biologie-horticultur%C4%83-tehnologia-prelucr%C4%83rii-produselor

2. Boruzi A.I., Nour V. 2017. Extracts of herbs and spices as natural antioxidants for Improving the functional value of meat products. Analele Universităţii din Craiova, seria Biologie, Horticultură, Tehnologia Prelucrării Produselor Agricole, Ingineria Mediului, vol. XXII (LVIII), 25-32. http://horticultura.ucv.ro/horticultura/sites/default/files/horticultura/Reviste/Analele/2017/anale2017_sectiuneai_pp_1_340.pdf

3. Tuțulescu F., Boruzi A.I., Nour V. 2019. Antibacterial activity of walnut leaves and sweet cherry stems in cooked pork patties. South Western Journal of Horticulture, Biology and Environment, Vol.10, No.2, pp.65-75 / art.e19105. http://biozoojournals.ro/swjhbe/v10n2.html

Articles communicated at national scientific sessions

1. Boruzi A.I., Nour V. 2018. Effect of adding walnut leaf powder as natural antioxidant in cooked pork patties. Scientific Conference of Doctoral Schools – Perspectives and challenges in doctoral research, SCDS-UDJG 2018, The 6th Edition, Galaţi, 7th-8th of June 2018 – lucrarea a fost distinsă cu premiul al III-lea.

2. Boruzi A.I., Nour V. 2019. Efficacy of the walnut leaf and sweet cherry stem extracts

as natural antioxidants in raw pork patties during frozen storage. Scientific Conference of Doctoral Schools – Perspectives and challenges in doctoral research, SCDS-UDJG 2019, The 7th Edition, Galaţi, 13th-14th of June 2019 – lucrarea a fost distinsă cu mențiune.

3. Boruzi A.I., Nour V. 2020. Effect of chitosan edible coating enriched with extracts from walnut leaves and sweet cherry stems on the quality of precooked pork patties during cold storage. Scientific Conference of Doctoral Schools – Perspectives and challenges in doctoral research, SCDS-UDJG 2020, The 8th Edition, Galaţi, 18th-19th of June 2020 – lucrarea a fost distinsă cu mențiune.

BIBLIOGRAPHY

1. Fernández-Ginés, J. M., Fernández-López, J., Sayas-Barberá, E., & Pérez-Alvarez, J. A. (2005). Meat products as functional foods: A review. Journal of food science, 70(2), R37-R43.
2. Prado, I. N., Campo, M. M., Muela, E., Valero, M. V., Catalan, O., Olleta, J. L., & Sañudo, C. (2015). Effects of castration age, protein level and lysine/methionine ratio in the diet on colour, lipid oxidation and meat acceptability of intensively reared Friesian steers. animal, 9(8), 1423-1430.
3. Maqsood, S., & Benjakul, S. (2011a). Comparative studies on molecular changes and pro-oxidative activity of haemoglobin from different fish species as influenced by pH. Food Chemistry, 124(3), 875-883.
4. Kumar, Y., Yadav, D. N., Ahmad, T., & Narsaiah, K. (2015). Recent trends in the use of natural antioxidants for meat and meat products. Comprehensive Reviews in Food Science and Food Safety, 14(6), 796-812.
5. Faustman, C., Sun Q., Mancini Q., Suman, S.P. (2010). Myoglobin and lipid oxidation interactions: Mechanistic bases and control. Meat Science, 86, 86–94.
6. Maisuthisakul, P., Suttajit, M., & Pongsawatmanit, R. (2007). Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. Food chemistry, 100(4), 1409-1418.
7. Artés, F., Gómez, P. A., & Artés-Hernández, F. (2007). Physical, physiological and microbial deterioration of minimally fresh processed fruits and vegetables. Food Science and Technology International, 13(3), 177-188.
8. Tanzadehpanah, H., Asoodeh, A., & Chamani, J. (2012). An antioxidant peptide derived from Ostrich (Struthio camelus) egg white protein hydrolysates. Food research international, 49(1), 105-111.
9. Asoodeh, A., Azam, A. G., & Chamani, J. (2012). Identification and characterization of novel antibacterial peptides from skin secretions of Euphlyctis cyanophlyctis. International Journal of Peptide Research and Therapeutics, 18(2), 107-115.
10. Hemeda, H. M., & Klein, B. P. (1990). Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. Journal of Food Science, 55(1), 184-185.
11. Ponce, M. A., Scervino, J. M., Erra-Balsells, R., Ocampo, J. A., & Godeas, A. M. (2004). Flavonoids from shoots and roots of Trifolium repens (white clover) grown in presence or absence of the arbuscular mycorrhizal fungus Glomus intraradices. Phytochemistry, 65(13), 1925-1930.
12. Sebranek, J. G., Sewalt, V. J. H., Robbins, K., & Houser, T. A. (2005). Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. Meat science, 69(2), 289-296.
13. Muchuweti, M., Kativu, E., Mupure, C. H., Chidewe, C., Ndhlala, A. R., & Benhura, M. A. N. (2007). Phenolic composition and antioxidant properties of some spices. American Journal of Food Technology, 2(5), 414-420.
14. Ozsoy, N., Candoken, E., & Akev, N. (2009). Implications for degenerative disorders: Antioxidative activity, total phenols, flavonoids, ascorbic acid, β-carotene and β-tocopherol in Aloe vera. Oxidative medicine and cellular longevity, 2(2), 99-106.
15. Wojdyło, A., Oszmiański, J., & Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. Food chemistry, 105(3), 940-949.
16. Lupea, A. X., Pop, M. A. R. I. A. N. A., & Cacig, S. V. E. T. L. A. N. A. (2008). Structure-radical scavenging activity relationships of flavonoids from Ziziphus and Hydrangea extracts. Rev Chim, 59(3), 309-13.
17. Gheldof, N., & Engeseth, N. J. (2002). Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. Journal of agricultural and food chemistry, 50(10), 3050-3055.
18. Brown, J. E., & Kelly, M. F. (2007). Inhibition of lipid peroxidation by anthocyanins,

anthocyanidins and their phenolic degradation products. European Journal of Lipid Science and Technology, 109(1), 66-71.
19. Tang, S. Z., Kerry, J. P., Sheehan, D., Buckley, D. J., & Morrissey, P. A. (2001). Antioxidative effect of dietary tea catechins on lipid oxidation of long-term frozen stored chicken meat. Meat Science, 57(3), 331-336.
20. Matuska, M., Lenart, A., & Lazarides, H. N. (2006). On the use of edible coatings to monitor osmotic dehydration kinetics for minimal solids uptake. Journal of Food Engineering, 72(1), 85-91.
21. Guilbert, S., Gontard, N., & Gorris, L. G. (1996). Prolongation of the shelf-life of perishable food products using biodegradable films and coatings. LWT-food science and technology, 29(1-2), 10-17.
22. Debeaufort, F., Quezada-Gallo, J. A., & Voilley, A. (1998). Edible films and coatings: tomorrow's packagings: a review. Critical Reviews in Food Science, 38(4), 299-313.
23. Krochta, J. M., & Mulder-Johston, C. (1997). Food Technology: Edible and Biodegradable Polymer Films Challenges and Opportunities (Scientific Status Summary) 51 (2).
24. Pothakamury, U. R., & Barbosa-Cánovas, G. V. (1995). Fundamental aspects of controlled release in foods. Trends in food science & technology, 6(12), 397-406.
25. Glicksman, M. (1983). Gum arabic (Gum acacia). Food hydrocolloids, 2, 7-29.
26. Whistler, R. L., & Daniel, J. R. (1985). Carbohydrates in food chemistry. Academic Press, New York.
27. Nisperos-Carriedo, M. O. (1994). Edible coatings and films based on polysaccharides. Edible coatings and films to improve food quality, 1, 322-323.
28. Ben, A., & Kurth, L. B. (1995). Edible film coating for meat cuts and primal. Meat 95, The Australian Meat Industry Research Conference.
29. Fernandez-Saiz, P., Lagaron, J. M., & Ocio, M. J. (2009). Optimization of the biocide properties of chitosan for its application in the design of active films of interest in the food area. Food Hydrocolloids, 23(3), 913-921.
30. Zivanovic, S., Chi, S., & Draughon, A. F. (2005). Antimicrobial activity of chitosan films enriched with essential oils. Journal of food science, 70(1), M45-M51.
31. Ziani, K., Fernández-Pan, I., Royo, M., & Maté, J. I. (2009). Antifungal activity of films and solutions based on chitosan against typical seed fungi. Food Hydrocolloids, 23(8), 2309-2314.
32. Arvanitoyannis, I. S. (2008). The use of chitin and chitosan for food packaging applications. In Environmentally compatible food packaging (pp. 137-158). Woodhead Publishing.
33. Sebti, I., Martial-Gros, A., Carnet-Pantiez, A., Grelier, S., & Coma, V. (2005). Chitosan polymer as bioactive coating and film against Aspergillus niger contamination. Journal of food science, 70(2), M100-M104.
34. Coma, V., Martial-Gros, A., Garreau, S., Copinet, A., Salin, F., & Deschamps, A. (2002). Edible antimicrobial films based on chitosan matrix. Journal of food science, 67(3), 1162-1169.
35. Coma, V., Deschamps, A., & Martial-Gros, A. (2003). Bioactive packaging materials from edible chitosan polymer—antimicrobial activity assessment on dairy-related contaminants. Journal of food science, 68(9), 2788-2792.
36. Durango, A. M., Soares, N. F. F., & Andrade, N. J. (2006). Microbiological evaluation of an edible antimicrobial coating on minimally processed carrots. Food control, 17(5), 336-341.
37. Han, C., Zhao, Y., Leonard, S. W., & Traber, M. G. (2004). Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (Fragaria× ananassa) and raspberries (Rubus ideaus). Postharvest Biology and Technology, 33(1), 67-78.
38. Park, S. I., Daeschel, M. A., & Zhao, Y. (2004). Functional properties of antimicrobial lysozyme-chitosan composite films. Journal of Food Science, 69(8), M215-M221.
39. Ribeiro, C., Vicente, A. A., Teixeira, J. A., & Miranda, C. (2007). Optimization of edible coating composition to retard strawberry fruit senescence. Postharvest Biology and

Technology, 44(1), 63-70.
40. Vásconez, M. B., Flores, S. K., Campos, C. A., Alvarado, J., & Gerschenson, L. N. (2009). Antimicrobial activity and physical properties of chitosan–tapioca starch based edible films and coatings. Food Research International, 42(7), 762-769.
41. Labuckas, D. O., Maestri, D. M., Perello, M., Martinez, M. L., & Lamarque, A. L. (2008). Phenolics from walnut (Juglans regia L.) kernels: Antioxidant activity and interactions with proteins. Food Chemistry, 107(2), 607-612.
42. Samaranayaka, A. G., John, J. A., & Shahidi, F. (2008). Antioxidant activity of English walnut (Juglans regia L.). Journal of Food Lipids, 15(3), 384-397.
43. Oliveira, I., Sousa, A., Ferreira, I. C., Bento, A., Estevinho, L., & Pereira, J. A. (2008). Total phenols, antioxidant potential and antimicrobial activity of walnut (Juglans regia L.) green husks. Food and chemical toxicology, 46(7), 2326-2331.
44. Stampar, F., Solar, A., Hudina, M., Veberic, R., & Colaric, M. (2006). Traditional walnut liqueur–cocktail of phenolics. Food chemistry, 95(4), 627-631.
45. Pereira, J. A., Oliveira, I., Sousa, A., Ferreira, I. C., Bento, A., & Estevinho, L. (2008). Bioactive properties and chemical composition of six walnut (Juglans regia L.) cultivars. Food and chemical toxicology, 46(6), 2103-2111.
46. Rahimipanah, M., Hamedi, M., & Mirzapour, M. (2010). Antioxidant activity and phenolic contents of Persian walnut (Juglans regia L.) green husk extract. Afr. J. Food Sci. Technol, 1(4), 105-111.
47. Cosmulescu, S. N., Trandafir, I., Achim, G., Mihai, B. O. T. U., Baciu, A., & Gruia, M. (2010). Phenolics of green husk in mature walnut fruits. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 38(1), 53-56.
48. Cosmulescu, S. N., Baciu, A., Achim, G., Mihai, B. O. T. U., & Trandafir, I. (2009). Mineral composition of fruits in different walnut (Juglans regia L.) cultivars. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 37(2), 156-160.
49. Cosmulescu, S. N., Trandafir, I., Achim, G., & Baciu, A. (2011). Juglone content in leaf and green husk of five walnut (Juglans regia L.) cultivars. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 39(1), 237-240.
50. Hooman, N., Mojab, F., Nickavar, B., & Pouryousefi-Kermani, P. (2009). Diuretic effect of powdered Cerasus avium (cherry) stems on healthy volunteers. Pakistan Journal of Pharmaceutical Sciences, 22(4), 381-383.
51. Di Cagno, R., Surico, R. F., Minervini, G., Rizzello, C. G., Lovino, R., Servili, M., ... & Gobbetti, M. (2011). Exploitation of sweet cherry (Prunus avium L.) puree added of stem infusion through fermentation by selected autochthonous lactic acid bacteria. Food Microbiology, 28(5), 900-909.
52. Ademović, Z., Hodžić, S., Zahirović, Z. H., Husejnagić, D., Džananović, J., Šarić-Kundalić, B., & Suljagić, J. (2017). Phenolic compounds, antioxidant and antimicrobial properties of the wild cherry (Prunus avium L.) stem. Acta Periodica Tehnologica, 48, 1-13.
53. Bastos, C., Barros, L., Dueñas, M., Calhelha, R. C., Queiroz, M. J. R., Santos-Buelga, C., & Ferreira, I. C. (2015). Chemical characterisation and bioactive properties of Prunus avium L.: The widely studied fruits and the unexplored stems. Food chemistry, 173, 1045-1053.
54. Martínez, M. L., Labuckas, D. O., Lamarque, A. L., & Maestri, D. M. (2010). Walnut (Juglans regia L.): genetic resources, chemistry, by-products. Journal of the Science of Food and Agriculture, 90(12), 1959-1967.
55. Carvalho, K. C. C., Mulinari, D. R., Voorwald, H. J. C., & Cioffi, M. O. H. (2010). Chemical modification effect on the mechanical properties of hips/coconut fiber composites. BioResources, 5(2), 1143-1155.
56. Solar, A., Colarič, M., Usenik, V., & Stampar, F. (2006). Seasonal variations of selected flavonoids, phenolic acids and quinones in annual shoots of common walnut (Juglans regia L.). Plant Science, 170(3), 453-461.
57. Jakopic, J., Colaric, M., Veberic, R., Hudina, M., Solar, A., & Stampar, F. (2007). How much do cultivar and preparation time influence on phenolics content in walnut liqueur?. Food Chemistry, 104(1), 100-105.

58. Aithal, K. B., Kumar, S. M., Rao, N. B., Udupa, N., & Rao, S. B. (2009). Juglone, a naphthoquinone from walnut, exerts cytotoxic and genotoxic effects against cultured melanoma tumor cells. Cell biology international, 33(10), 1039-1049.
59. Verma, S. P., & Sahoo, J. (2000). Improvement in the quality of ground chevon during refrigerated storage by tocopherol acetate preblending. Meat science, 56(4), 403-413.
60. Faustman, C., & Cassens, R. G. (1990). The biochemical basis for discoloration in fresh meat: a review. Journal of Muscle Foods, 1(3), 217-243.
61. Rhee, K. S., & Ziprin, Y. A. (2001). Pro-oxidative effects of NaCl in microbial growth-controlled and uncontrolled beef and chicken. Meat Science, 57(1), 105-112.
62. Kagan, V. E. (2018). Lipid peroxidation in biomembranes. CRC press.
63. Al-Kahtani, H. A., Abu-Tarboush, H. M., Bajaber, A. S., Atia, M., Abou-Arab, A. A., & El-Mojaddidi, M. A. (1996). Chemical changes after irradiation and post-irradiation storage in tilapia and Spanish mackerel. Journal of food science, 61(4), 729-733.
64. Teets, A. S., Sundararaman, M., & Were, L. M. (2008). Electron beam irradiated almond skin powder inhibition of lipid oxidation in cooked salted ground chicken breast. Food Chemistry, 111(4), 934-941.
65. Xu, Y. X., Kim, K. M., Hanna, M. A., & Nag, D. (2005). Chitosan–starch composite film: preparation and characterization. Industrial crops and Products, 21(2), 185-192.
66. Rodriguez-Sanchez, Dolores., & Rha, C. (1981). Chitosan globules. International Journal of Food Science & Technology, 16(5), 469-479.
67. Caner, C., Vergano, P. J., & Wiles, J. L. (1998). Chitosan film mechanical and permeation properties as affected by acid, plasticizer, and storage. Journal of food science, 63(6), 1049-1053.
68. Rhim, J. W., Weller, C. L., & Ham, K. S. (1998). Characteristics of chitosan films as affected by the type of solvent acid. Food Science and Biotechnology, 7(4), 35-40.
69. Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D., & Richardson, R. I. (2006). Flavour perception of oxidation in beef. Meat Science, 72(2), 303-311.
70. Ponce, A. G., Roura, S. I., del Valle, C. E., & Moreira, M. R. (2008). Antimicrobial and antioxidant activities of edible coatings enriched with natural plant extracts: in vitro and in vivo studies. Postharvest biology and technology, 49(2), 294-300.