University „Dunărea de Jos” of Galaţi

Doctoral School of Fundamental Sciences and Engineering

DOCTORAL THESIS

POSSIBILITIES FOR THE USE OF SOME SECONDARY PRODUCTS OF FRUIT AND FRUIT PROCESSING TO INCREASE THE FUNCTIONALITY OF FOODS PRODUCTS

(Doctoral thesis summary)

PhD Student,

Corbu Alexandru Radu

Scientific leader,

Proff. dr. ing. Nour Violeta

Series I 7: FOOD ENGINEERING Nr.13

GALAŢI

2020

University „Dunărea de Jos” of Galaţi

Doctoral School of Fundamental Sciences and Engineering

DOCTORAL THESIS

POSSIBILITIES FOR THE USE OF SOME SECONDARY PRODUCTS OF FRUIT AND FRUIT PROCESSING TO INCREASE THE FUNCTIONALITY OF FOODS PRODUCTS

(Doctoral thesis summary)

PhD Student

Corbu Alexandru Radu

Scientific leader, Prof univ.dr.ing. Nour Violeta
Scientific reviewers Prof univ.dr.ing. Râpeanu Gabriela
Prof.univ.dr. Mudura Elena
Prof.univ.dr. Oroian Mircea

Series I 7: FOOD ENGINEERING Nr.13

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

I. SCIENTIFIC GOALS OF THE PHD THESIS

Industrial processing of fruits and vegetables generates huge quantities of by-products and wastes as peels, seeds, kernels, cakes, marc, unripe or damaged fruits and vegetables. Researches performed in the last 20 years revealed the fact that many by-products could serve as a source of bioactive compounds potentially valuable such as minerals, vitamins, sugars, carotenoids, fibers, phenolic compounds, aromatic compounds, etc. However, majority of fruits and vegetables by-products and wastes are not yet exploited.

Industrial processing of tomatoes generates huge quantities of by-products with a total of 10-30% of the total quantity of the tomatoes that are being processed. These by-products, which include seeds, peel and pulp, are a valuable source of functional compounds such as phenolic compounds, fibers and minerals and can be used for developing foods with added value due to their nutritional and functional characteristics. Tomatoes by-products generally contain a high level of dietary fibers, proteins, fats and minerals, but also a high content of carotenoids. Tomatoes processing industry generates huge quantities of by-products representing 10-30%

Sea buckthorn fruits are a rich source of bioactive compounds that can contribute to the health benefits claimed and proved by the juice and oil of sea buckthorn fruits. Nutrition value of the sea buckthorn and its pharmacological effects are determined by the high content of vitamins and secondary plant metabolites. Sea buckthorn pomace is a secondary product produced during the juice extraction, and it is formed by pulp, seeds and peels. This contains high quantities of vitamins, flavonoids, carotenoids and special fatty acids. Utilization of these by-products as raw material for the extraction of benefic components increased în the last decades.

These few considerations show the importance and opportunity of studies for the recovery of the by-products and wastes that are obtained during the industrial processing of the tomatoes and sea buckthorn as supplements for animal feed but also for the extraction of bioactive compounds, aiming to obtain in the end foods with high functionality enriched with bioactive antioxidant compounds.

Moreover, the studies developed in these work started from the hypothesis that an increase of the carotenoids content in foods, especially in products with a high level of fatty acids, including PUFA, will contribute to the antioxidant protection of these foods, resulting in the increase of their oxidative and thermal stability.

In the context of current research, this PhD thesis has set the following specific scientific objectives:

→ Characterization of the by-products resulting during the industrial processing of tomatoes in terms of nutritional and bioactive compounds content;

→ Characterization of the by-products resulting during the industrial processing of sea buckthorn fruits in terms of nutritional and bioactive compounds content;

→ Study the effects of direct extraction of carotenoids from the dehydrated tomatoes by-products on the stability and characteristics of some vegetable oils;

→ Enriching the vegetable oils with carotenoids obtained through direct extraction from the secondary by-products resulted during the processing of the white sea buckthorn, effect of this enrichment upon the physicochemical characteristics and oxidative and thermal stability of the vegetable oils;

→ Effect produced by supplementation of the diet of laying hens with by-products resulted during the tomatoes processing upon the laying performances of the hens, eggs quality, yolk carotenoids content and eggs color;

→ Studies regarding the simultaneous inclusion of flaxseeds and tomatoes processing by-products in the diet of laying hens for enriching the eggs with n-3 PUFA and carotenoids and increase the oxidative stability of the so obtained “designer eggs”.

II. DOCUMENTARY STUDY

CHAPTER 1

AGROINDUSTRIAL WASTES AND THEIR POTENTIAL RECOVERY

Food industry produces a high quantity of wastes of fruits and vegetables. These generally contain big quantities of solid substances (SS) and need an important usage of biochemical oxygen demand (BOD) and chemical oxygen demand (COD), which is influencing the possible recovery solutions and the cost of the treatments. Wastes consist especially in hydrocarbon and relatively small quantities of proteins and lipids, having a humidity of 80-90%. Finally, waste waters contain dissolved compounds, pesticides, herbicides and detergents [1].

1.1. Recovery of secondary products from processing foods

Secondary products, waste and effluents that result after processing fruits and vegetables contain big quantities of proteins, sugars, lipids and aromatic and aliphatic compounds and thus can be cheap and abundant sources of good quality chemical products and biomaterials. Indeed, after specific treatments using physical and biologic agents, followed by characteristic recovery procedures, these can be natural antioxidants with added value, antimicrobial agents, vitamins etc., as well as macromolecules (such as cellulose, starch, lipids, proteins, enzymes and plants pigments) that present a special interest for pharmaceutical, cosmetics and food industries.

1.2. Recovery of secondary products and wastes resulting from industrial processing of fruits and vegetables

In the last 25 years, processing of fruits, vegetables and cereals has considerably grown as a consequence of the epidemiological studies tying the dietary consumption of vegetable fibers, from cereals and fruits, with the decrease of mortality due to cancer and cardiovascular diseases [2]. Processing fruits and vegetables generates big quantities of residual and secondary products (20-60% w/w of the fruits and vegetables that are processed) [3].

By-products and wastes of fruits and vegetables (peels, seeds and pulp that are not used during the stages of the production process), which are in their main part thrown away, are causing not only the pollution of the environment but are also sources of bio components [4]. In general vegetal residues are a good natural source of carbohydrates, polysaccharides, vitamins, minerals, antioxidants [5] while secondary products can have a high content of bioactive compounds [4]. Kernels and oils that can be recovered from those are rich in various compounds such as tococromanols, essential fatty acids, phytosterols and squalene [6] while apples pomace is a good source rich in pectines, carbohydrates, fibers and minerals [7]. Recovery of the high value compounds allows the usage of those as good additives and/or nutraceutics.

CHAPTER 2

RECOVERY OF THE BY-PRODUCTS FROM TOMATOES PROCESSING

2.1. Compositional characterization of by-products resulted after processing tomatoes

Tomatoes represent worldwide the second largest vegetable crop after potatoes, with an annual production of 100 million tones fresh fruits produced in 144 countries. In 2013, world production of tomatoes was around 163 million tones [8].

Industrial processing of tomatoes produces big quantities of wastes, respectively 20-50 kg/ton which could be used for producing antioxidants [9, 10]. After processing tomatoes, 3-7% of their weight, meaning the peels, a part of the pulp and seeds became wastes usually used for animal feed or even thrown away. Secondary products resulted after tomatoes processing represent a rich source of nutritive substances and active biologic compounds: carotenoids, proteins, phenolic compounds, minerals and oils.

2.2. Using the by-products resulted after tomatoes processing as animal feed

Dry tomatoes pomace is an excellent source of tocopherol (vitamin E), which is used as antioxidant for broiler meat. Secondary tomatoes products are usually included in the ruminants' diet due to their high content of fibers. Even though lycopene seems to attract a limited attraction as egg yolk pigment, several studies investigated the effect of diet enriched with lycopene for laying hens upon the performance and quality of the eggs.

2.3. Lycopene and β-carotene extraction from by-products resulted after tomatoes processing

Several studies were conducted in order to increase the recovery of lycopene from secondary products resulted after processing tomatoes. Some of the suggested solutions are processing at high hydrostatic pressure [11], enzymatic treatment with cellulase and pectinase [12, 13] or surfactants [14] and extraction with supercritical fluids [15, 16]. Still, until now the usual extraction technologies seems not to allow an efficient recovery of carotenoids [14].

2.4. Enriching oils with carotenoids through direct extraction of by-products from tomatoes processing

Recent studies focused on enriching oils using natural antioxidants from some plant species, known of having antioxidant properties [17]. The activity of these plant extracts was attributed to the presence of flavone compounds, phospholipids, tocopherols and ascorbic acid [18]. Can be also used antioxidant compounds from residual sources for increasing the stability of vegetable oils [19]. There are not enough studies regarding the incorporation of brute extracts or of the entire residual vegetable material as antioxidant in oils.

2.5. Usage of secondary products from tomatoes processing as food ingredients

Some researchers in food domain suggested avoiding the extraction of lycopene and other carotenoids and the usage through the direct recycling of the by-products as food ingredients. Thus, dehydrated tomato peels were added in dry sausages, hamburgers, minced poultry processed under high pressure, extruded products type snack-foods, ketchup, bread, muffins crackers, spread cheese, pasta etc.

CHAPTER 3

RECOVERY OF BY-PRODUCTS FROM SEA BUCKTHORN PROCESSING

3.1. The nutritional potential of sea buckthorn fruits

White sea buckthorn is one of the shrubs of great value both as spontaneous flora and cultivated varieties, due to importance of its fruits in pharmaceutical and food industry, silviculture, cosmetics and ornamental plant.

Sea buckthorn fruits are rich in carbohydrates, soluble vitamins, proteins, fats, antioxidants (vitamins C and E, β-carotene and lycopene), essential fatty acids, amino acids, phytosterols, flavonoids, minerals (iron, calcium etc.) [20, 21]. Fruits are used in many industrial products, including drugs and medicinal plants, for treatment of cancer, cardiac diseases, ulcers, hepatic disorders, burns and cerebral dysfunctions.

The most valuable components of sea buckthorn berries are their oils. Both seeds and pulp have a high content of lipids, including tocopherols, tocotrienols, carotenoids and also the fatty acids families omega-3 and omega-6 [22].

3.2. Processing of sea buckthorn fruits

Products on the market include oils, juices and food additives for candies, cosmetics and shampoos [23].

Sea buckthorn fruits can be used for baking pies, jams, lotions and liqueurs. Juice or pulp have also potential usage in foods and drinks [24]. Due to its high nutritional value and the increasing market demand it was suggested that sea buckthorn fruits can be tested for usage in order to increase the nutritional value and functionality of various foods such as: jams, juices, drinks.

3.3. Recovery of the by-products from the processing of sea buckthorn fruits

Juice extraction from sea buckthorn berries results in obtaining of a pressed waste cake that contains especially flavones [25]. Sea buckthorn pomace is a byproduct that results after juice extraction and it is formed by pulp, seeds and peels. During the last decades, the interest in using these by-products as raw material for other processes in order to extract their benefic compounds was continuously growing [26]. Besides the waste reduction, the purpose for usage of these by-products is the extraction of their antioxidant compounds.

III. EXPERIMENTAL RESULTS

CHAPTER 4

NUTRITIONAL VALUE AND THE CONTENT OF BIOLOGICALLY ACTIVE COMPOUNDS IN BY-PRODUCTS RESULTED FROM TOMATOES PROCESSING

4.1. Study opportunity

The purpose of this study was to determine the content of different nutritional substances and biologically active compounds (carotenoids, polyphenols, amino acids and fatty acids) of the by-products resulted after the industrial processing of tomatoes. Results of this study might allow the development of a new way for recycling this valuable secondary product.

4.2. Materials and methods of analyses

Two samples (100 kg) of industrial by-products from tomatoes processing (mix of peels and seeds) were collected from Leader International S.A., a commercial society that is involved in fruits and vegetables processing in Caracal. After collection by-products were packed in plastic bags and were frozen at -25^{o}C. Tomatoes by-products were dried using an industrial drier (Blue Spark Systems S.R.L., Romania) with hot air at 60^{o}C. After drying, the material was grinded using an electric grinder and then was passed through a sieve of 1 mm. Samples were analyzed for determining humidity, brute protein, brute fats and fiber content, total phenolic compounds content, falvonoids, lycopene and β-carotene, and also the antioxidant activity. Phenolic profile, as well as amino acids and fatty acids profiles were determined using chromatographic methods. The content of minerals was determined using inductively coupled plasma mass spectrometry.

4.3. Results and discussions

4.3.1. Chemical composition

Dehydrated tomatoes by-products, approximately 22.2% seeds and 77.8% rests of cellulose and peels, were characterized from the macronutrient content point of view (proteins, fats, fibers and ash), results being presented in Table 4.1.

Table 4.1. General chemical composition of dehydrated tomatoes by-products

Component (g/kg)	Dehydrated tomato by-products (peels+seeds)
Dry substance	946.5 ± 13.2
Proteins	176.2 ± 7.4
Lipids	21.9 ± 2.0
Fibers	524.4 ± 18.3
Ash	42.1 ± 3.6

4.3.2. Amino acids content

Results regarding the amino acids content of dehydrated tomato by-products are shown in Table 4.2. Glutamic acid, a non-essential amino acid, was the most abundant in the dehydrated tomatoes by-products (72.1 g/kg). In this study were determined 8 essential amino acids: leucine, isoleucine, lysine, methionine, phenylalanine, threonine, arginine and valine, representing 34.2% of the total amino acids content. From essential amino acids, the most abundant was leucine (10.7 g/kg), followed by lysine (8.85 g/kg) and isoleucine (6.87 g/kg), while the methionine content was very low (2.7 g/kg). Previous studies shown that in tomato peels was generally determined a lower quantity of essential amino acids than in seeds [9], which means that the amino acids profile will depend on that ratio between peels/seeds in the secondary product.

Table 4.2. Content of amino acids present in dehydrated tomatoes by-products (g/kg)

Amino acids	Dehydrated tomato by-products (peels+seeds)
Aspartic acid	15.7 ± 0.4
Glutamic acid	72.1 ± 3.2
Serine	1.7 ± 0.1
Glycine	6.3 ± 0.2
Treonină	5.5 ± 0.2
Arginine	14.6 ± 0.6
Alanine	7.1 ± 0.3
Tyrosine	6.9 ± 0.4
Valine	5.4 ± 0.3
Phenylalanine	6.1 ± 0.4
Isoleucine	6.9 ± 0.2
Leucine	10.7 ± 0.4
Lysine	8.8 ± 0.3
Cysteine	2.3 ± 0.1
Methionine	2.7 ± 0.2
Total amino acids	172.4 ± 6.7

4.3.3. Fatty acids content

Fatty acids were determined in dehydrated tomato by-products using gas chromatography, concentrations are being shown in Table 4.3.

Results shown that linoleic acid is the most present (51.91% of total fatty acids), followed by oleic acid (18.50%), while palmitic acid was the principal saturated acid (16.32%). Unsaturated fatty acids represent 77.04% of total fatty acid content while saturated fatty acids represent 22.72% which is showing that fatty acids are dominant in dehydrated tomato by-products. In human nutrition a big ratio between n-6:n-3 PUFA is known as a risk factor for cancer incidence and coronary diseases [27]. For dehydrated tomato by-products this ratio was of 12.56:1, lower than the ratio of 15:1 reported by Simopoulos (2002) [28] for the typical occidental diet, but higher than the ratio of 10:1 present in typical American diet [29].

Table 4.3. Fatty acids profile in dehydrated tomato by-products (g of fatty acid per 100 g total fatty acids).

Fatty acids		Dehydrated tomato by-products (peels+seeds)
Myristic	C 14:0	0.41 ± 0.02
Pentadecanoic	C 15:0	0.09 ± 0.03
Pentadecenoic	C 15:1	0.09 ± 0.02
Palmitic	C 16:0	16.32 ± 0.65
Palmitoleic	C 16:1	0.64 ± 0.03
Heptadecanoic	C 17:0	0.19 ± 0.01
Heptadecenoic	C 17:1	0.52 ± 0.02
Stearic	C 18:0	5.43 ± 0.34
cis-Oleic	C 18:1	18.50 ± 0.83
cis-Linoleic	C 18:2n6	51.91 ± 1.91
γ-Linoleic	C 18:3n6	Nd
α-Linoleic	C 18:3n3	3.35 ± 0.24
Octadecatetraenoic	C18:4n3	0.48 ± 0.03
Eicosadienoic	C20(2n6)	0.15 ± 0.01
Eicosatrienoic	C20(3n6)	0.07 ± 0.01
Docosadienoic	C22(2n6)	0.39 ± 0.02
Docosatrienoic	C22(3n6)	0.55 ± 0.03
Docosatrienoic	C22(3n3)	0.13 ± 0.01
Eicosapentaenoic	C20(5n3)	0.26 ± 0.01
Lignoceric	C 24:0	0.29 ± 0.02
Other fatty acids		0.22 ± 0.01
Fatty acids profile		
Saturated fatty acids (SFA)		22.72 ± 0.94
Monounsaturated fatty acids (MUFA)		19.75 ± 0.82
Polyunsaturated fatty acids (PUFA)		57.29 ± 2.13
n-3		4.22 ± 0.28
n-6		53.07 ± 1.76
n-6/n-3		12.57 ± 0.48

4.3.4. Minerals content

Results of the analysis regarding the mineral content in dehydrated tomato by-products are shown in Table 4.4. Among macronutrients, potassium was in the biggest concentration (30301.7 mg/kg), followed by calcium (1318 mg.kg). In dehydrated tomato by-products, the content of sodium is quite big, which consequently is limiting its inclusion in birds' diet [9].

Table 4.4. Minerals content dehydrated tomatoes by-products (mg/kg).

Minerals	Dehydrated tomatoes by-products (peels+seeds)
Calcium	1318.5 ± 43.3
Magnesium	2109.7 ± 67.8
Potassium	30301.7 ± 588.1
Sodium	665.5 ± 33.5
Iron	56.3 ± 6.4

Manganese	13.5 ± 2.2
Copper	11.5 ± 2.6
Chromium	3.5 ± 1.3
Zinc	63.3 ± 5.1
Boron	19.5 ± 3.2

4.3.5. Total phenolic content, flavonoids, lycopene, β-carotene and free radicals scavenger activity

Total average content of phenolic compounds determined in dehydrated tomato by-products was 1229.5 mg GAE/kg (Table 4.5). An important part of the phenolic compounds is represented by flavonoids (415.3 QE/kg). Result is in accordance with previous researches that shown in tomato peels high concentration of flavonols, especially quercetin and kaempherol [30]. Dehydrated tomato by-products presented a high antioxidant activity (6.8 mmol Trolox/kg) (Table 4.6). A previous study also shown notable effect of tomato peels in free radicals scavenging activity and attributed this to the high content of lycopene and phenolic compounds [31].

Table 4.5. Total phenolic content, flavonoids, lycopene, β-carotene and free radical scavenger activity of dehydrated tomato by-products

Content	Dehydrated tomato by-products (peels+seeds)
Total phenolic content (mg GAE/kg)	1229.5 ± 55.5
Total flavonoids (mg QE/kg)	415.3 ± 18.2
Lycopene (mg/kg)	510.6 ± 21.1
β-Carotene (mg/kg)	95.6 ± 3.3
Antioxidant activity (mmol Trolox/kg)	6.8 ± 0.2

Regarding carotenoids profile, dehydrated tomato by-products have a high content of lycopene (105.38 mg/100g), together with β-carotene (9.50 mg/100g) and lutein (3.57 mg/100g) (Table 4.6).

Table 4.6. Carotenoids content in dehydrated tomato by-products

Carotenoids	Dehydrated tomato by-products (peels+seeds)
Astaxanthin (mg/kg)	0.076
Lutein (mg/kg)	3.57
Zeaxanthin (mg/kg)	0.78
Canthaxanthin (mg/kg)	0.27
Apocarotenal (mg/kg)	0.20
Lycopene (mg/kg)	105.38
Beta carotene (mg/kg)	9.50

4.3.6. Content of phenolic compounds

The most abundant phenolic acids quantified in dehydrated tomato by-products were ellagic acid (143.4 mg/kg) and clorogenic acid (76.3 mg/kg). Phenolic acids determined in

smaller concentrations were salicylic acid, gallic acid, vanillic acid, coumaric acid, syringic acid. Among flavonoids were detected and quantified only rutin and myricentin (Table 4.7).

Tableul 4.7. Content of phenolic compounds in dehydrated tomato by-products (mg/kg)

Phenolic compounds	Dehydrated tomato by-products (peels+seeds)
Gallic acid	17.1 ± 0.6
Catechin hydrate	Nd*
Vanillic acid	26.9 ± 1.1
Clorogenic acid	76.3 ± 2.8
Caffeic acid	Nd
Syringic acid	2.2 ± 0.1
Epicatechin	Nd
Coumaric acid	11.4 ± 0.5
Sinapic acid	Nd
Salicylic acid	66.9 ± 2.7
Rutin	29.2 ± 1.1
Ellagic acid	143.4 ± 5.9
Myricentin	63.7 ± 2.2
Trans-cinnamic acid	Nd
Quercetin	Nd

*Nd - undetected

4.4. Partial conclusions

Results of this study proved that dehydrated tomato by-products (peels and seeds) have a high nutritional value based on the content of essential amino acids, fatty acids and minerals, suggesting that these by-products have a substantial value as animal feed. However, their extremely high content of fibers is limiting their usage in poultry due to low digestibility and low contribution to metabolizable energy.

The high content of carotenoids present in dehydrated tomato by-products, coming especially from the peels faction, led to an increasing interest for lycopene and β-carotene extraction, because those are used on a large scale as food colorants, functional food ingredients or as functional food supplements or simply food supplements, pharmaceutics and cosmetics. However, in order to avoid lycopene extraction, which proved to be inefficient and expensive, direct inclusion of the dehydrated tomato by-products in foods could prove to be a way to use these wastes for obtaining new food products enriched in bioactive compounds.

Besides lycopene, by-products resulted from the industrial processing of tomatoes are rich in phenolic compounds with high antioxidant activity, which will help increasing food functionality in which these by-products will be added. A better knowledge of the composition of these by-products from industrial processing of tomatoes could lead to their transformation in products with higher value and to the improvement of tomato wastes management, thus increasing the economic performance of tomatoes processing and reducing the problems related to wastes elimination.

CHAPTER 5

NUTRITIONAL VALUE AND THE CONTENT OF BIOLOGICALLY ACTIVE COMPOUNDS IN BY-PRODUCTS RESULTED FROM SEA BUCKTHORN PROCESSING

5.1. Study opportunity

Fruits of sea buckthorn present lately a high interest due to the nutraceutic properties and their high content of antioxidant compounds. Juice and pulp of sea buckthorn fruits are often used as foods and drinks because are very rich in vitamins, carotenoids, flavonoids, tocopherols and other components with a benefic potential for health [32, 33]. Sea buckthorn fruits have an appreciable content of oil that contains, among other components, two essential fatty acids, linolenic (n-3) and linoleic (n-6). The high content of tocopherols, tocotrienols and carotenoids that can be found in this oil [34] gives antioxidant properties, shown by numerous studies performed both on humans and *in vitro* [35].

Sea buckthorn processing for juice extraction results in a high quantity of by-products, approximately 20% of the total weight of the fruits. These by-products are formed by pulp, seeds and peels and are recognized as rich in carotenoids, polyphenols, fatty acids and sterols [36, 37, 38]. This study had as goal to evaluate the nutritional properties, bioactives and antioxidants of dried sea buckthorn pomace in order to promote consumption and usage in the food industry of this secondary product that is an extremely valuable resource.

5.2. Materials and methods

Samples of sea buckthorn pomace were collected from Biocat Prod S.R.L., a commercial producer and processor of sea buckthorn fruits locate in Grădina, county of Constanța. Immediately after collection, samples were dried in an automatic industrial dryer with hot air flux at 60^{o}C (Blue Spark Systems S.R.L., Romania) and the dried material was transformed in powder.

Samples were analyzed for determining humidity, contents of brute protein, brute fat and fiber and also total content of phenolic compounds, flavonoids and antioxidant activity. Phenolic and carotenoids profile as well as amino acids and fatty acids profiles were determined using chromatographic methods. Content of minerals was determined by graphite furnace atomic absorbtion spectroscopy.

5.3. Results and discussions

5.3.1. Chemical composition

One of the main characteristics of sea buckthorn fruits is their high content of fat (Table 5.1). In contrast to other fruits, sea buckthorn fruits synthetize and accumulate lipids in all fruit parts so consequently it is possible to obtain three types of oils according to the extraction site, meaning pulp, seeds and peels [39]. However, due to the fact that is hard to separate seeds

from pulp, normally these two oils are not distinct and are generically called pulp oil and soft parts oil.

Table 5.1. Dry sea buckthorn pomace composition (g/kg)

Component	Dry sea buckthorn pomace
Dry substance	926.6 ± 11.8
Brute protein	148.9 ± 6.5
Brute fat	200.5 ± 5.3
Brute fiber	198.6 ± 8.9
Ash	18.4 ± 0.8

5.3.2. Amino acids content

Glutamic acid was in the biggest quantity (23.7 g/kg) in dry sea buckthorn pomace, followed by aspartic acid (17.2 g/kg) (Table 5.2). Essential amino acids represented 38.42% of the total amino acids content, the highest quantity being leucine (11.6 g/kg) followed by phenylalanine and lysine.

Table 5.2. Amino acids content in dry sea buckthorn pomace (g/kg)

Amino acids	Dry sea buckthorn pomace
Aspartic acid	17.2 ± 0.5
Glutamic acid	23.7 ± 1.1
Serine	8.5 ± 0.3
Glycine	5.1 ± 0.2
Threonine	5.2 ± 0.2
Arginine	13.1 ± 0.4
Alanine	6.8 ± 0.3
Tyrosine	4.4 ± 0.2
Valine	6.4 ± 0.3
Phenylalanine	7.9 ± 0.3
Isoleucine	7.1 ± 0.2
Leucine	11.6 ± 0.3
Lysine	7.2 ± 0.2
Cystine	1.5 ± 0.1
Methionine	4.7 ± 0.2
Total amino acids	130.4 ± 4.8

5.3.3. Fatty acids content

Dry sea buckthorn pomace had a low ratio between polyunsaturated fatty acids n-6/n-3 (PUFA) of 1.42 and a high concentration of monounsaturated fatty acids (MUFA) of 53.08% of total fatty acids content, as a result of high content of oleic and palmitic acids. These results are in accordance with other data shown in previous studies [40]. A higher dietary intake of palmitoleic acid is reducing cholesterol and triglycerides, improves the ratio between HDL and LDL and is inhibiting inflammatory processes [41, 42].

Table 5.3. Fatty acids profile in dry sea buckthorn pomace (% of total fatty acids content)

Fatty acids profile	
Saturated fatty acids (SFA)	31.18 ± 1.40
Monounsaturated fatty acids (MUFA)	53.08 ± 2.08
Polyunsaturated fatty acids (PUFA) from which:	15.70 ± 0.72
▪ n-3	6.48 ± 0.30
▪ n-6	9.22 ± 0.42
n-6/n-3	1.42 ± 0.12

5.3.4. Minerals content

Minerals that were taken into consideration in this study were calcium, iron, manganese, cooper and zinc (Table 5.4) Calcium content found in dry sea buckthorn pomace (724 mg/kg) was higher than the one reported in previous studies (40-100 mg/kg) in fresh fruits [43]. Similarly, iron (62.9 mg/kg), manganese (12.6 mg/kg) and zinc (22.3 mg/kg) levels determined were higher than those previously reported in fresh fruits [44]. However, results are in accordance with those reported by Sabir et al. (2005) [45] that found in dry fruits an iron and calcium content of 700-1250 mg/kg respectively 40-225 mg/kg.

Table 5.4. Minerals content in dry sea buckthorn pomace (mg/kg)

Minerals	Dry sea buckthorn pomace
Calcium	264.8 ± 15.6
Iron	62.9 ± 2.6
Manganese	12.6 ± 0.5
Cooper	8.3 ± 0.3
Zinc	22.3 ± 1.0

5.3.5. Total content of phenolic compounds

Sea buckthorn fruits are a rich source of phenolic compounds. Major groups of polyphenols identified in fruits are flavonols and condensed tannins [37, 46], compounds that are giving a very high antioxidant potential. However, the antioxidant capacity of sea buckthorn fruits is attributed to the combined action of ascorbic acid, polyphenols (phenolic acids and flavonoids) and carotenoids [47].

Table 5.5. Total content of phenolic compounds, flavonoids, carotenoids and antioxidant activity (ABTS) of dry sea buckthorn pomace

Component	Dry sea buckthorn pomace
Total phenolic content (mg GAE/kg)	2791.2 ± 26.6
Total flavonoids content (mg QE/kg)	482.5 ± 20.2
Total carotenoids content (mg/kg)	245.6 ± 11.5
Antioxidant activity (mmol Trolox/kg)	82.96 ± 3.6

5.3.6. Carotenoids content

Figure 5.1. represents a HPLC chromatogram at 450 nm of carotenoids from dry sea buckthorn pomace. The most important pigments were β-carotene (80.76 mg/kg) and zeaxanthin (69.60 mg/kg), but were also quantified other carotenoids such as lutein, astaxanthin, trans-β-apo-8'-carotenal, canthaxanthin and lycopene (Table 5.6).

Table 5.6. Carotenoids content of dry sea buckthorn pomace (mg/kg)

Carotenoids	Dry sea buckthorn pomace
Astaxanthin	4.78 ± 0.23
Lutein	6.96 ± 0.41
Zeaxanthin	69.60 ± 1.56
Canthaxanthin	1.36 ± 0.22
Trans-β-apo-8'-carotenal	2.36 ± 0.12
Lycopene	0.91 ± 0.06
β-Carotene	80.76 ± 3.58
Total carotenoids	166.73 ± 6.18

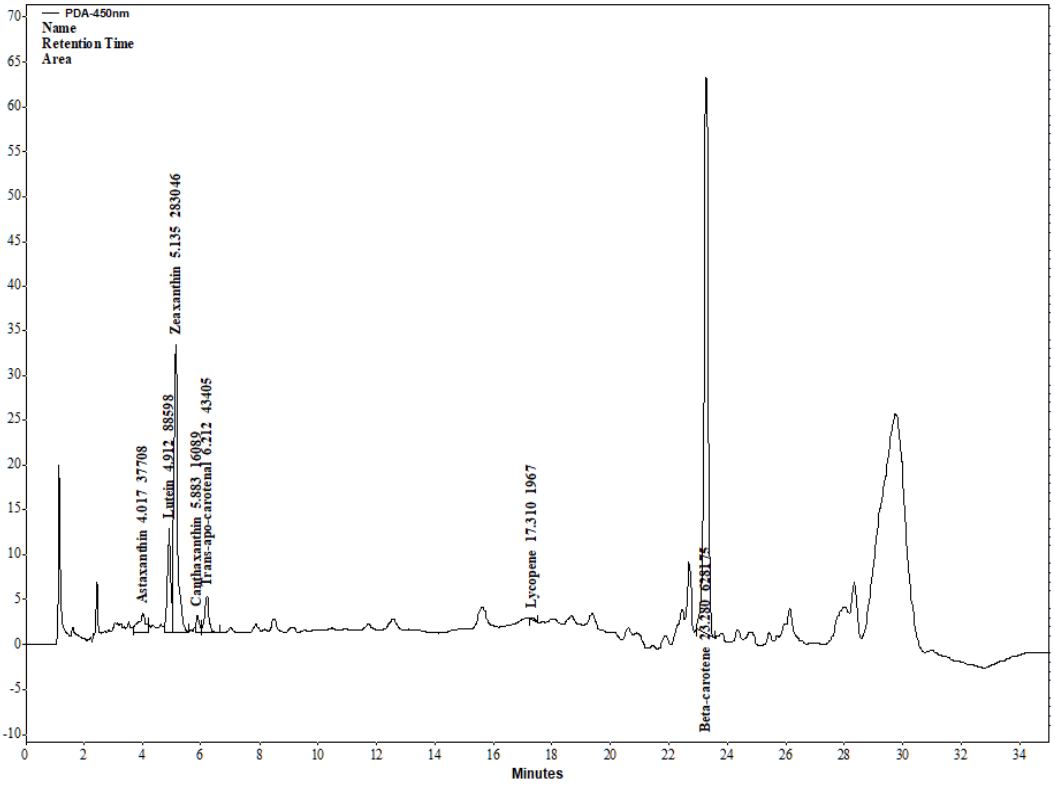

Figure 5.1. HPLC chromatogram of carotenoids from samples of dry sea buckthorn pomace.

5.4. Partial conclusions

Results of this study shown that sea buckthorn pomace has a high nutritional value given by its high content of fats and proteins, essential amino acids (38.42% of total amino acids content) and fatty acids profile characterized by the predominance of MUFA (15.7%) and the low ratio between n-6/n-3 fatty acids (1.42). These results suggest that sea buckthorn fuits pomace can be a valuable food ingredient or can be used as a nutritional supplement. Moreover, by-products of sea buckthorn fruits contain high levels of phenolic compounds and carotenoids and show a strong antioxidant activity. Content of bioactive compounds allows sea buckthorn pomace usage as functional food supplement and as a natural source of antioxidants in medical and pharmaceutical industry.

CHAPTER 6

EFECTS OF CAROTENOIDS EXTRACTED FROM DRY TOMATO BY-PRODUCTS ON THE STABILITY AND CHARACTERISTICS OF SOME VEGETABLE OILS

6.1. Study opportunity

In this study were used several types of vegetable oils as alternative solvent for obtaining enriched oils with carotenoids for various food usage. Were performed comparative studies between maceration, ultrasound assisted extraction (UAE) and microwave assisted extraction (MAE) regarding carotenoids extraction efficacy from wastes resulted from industrial tomatoes processing. Color and oxidative stability of the vegetable oils resulted after such extractions were also compared.

6.2. Materials and methods

Dehydrated tomato by-products, containing peels and seeds of ripped tomatoes, were obtained from Elio Monte Verde, a tomatoes processing unit from Caracal, Romania. 20 kg of waste were collected, dried with hot air at 60^{o}C, and then finely milled.

Three experiments were performed in order to investigate the influence of oil type, extraction method and concentration of dry tomato by-products on carotenoids extraction and vegetable edible oil oxidative stability after enriching them with carotenoids extracted from the secondary tomatoes products. In the first experiment, dried and milled samples of tomato waste were undergoing an ultrasound assisted extraction at a concentration of 5% (weight/volume) in extra virgin sunflower oil, unrefined corn oil, refined rape seeds oil, extra virgin olive oil, pomace olive oil, soy oil, refined sunflower oil, peanuts oil, rice oil, and grape seeds oil, at 20^{o}C for 50 minutes.

The second experiment had as goal to evaluate the influence of extraction method. Waste samples (5 g) were subjected to every extraction procedure: (a) ultrasound assisted extraction in 100 ml oil at 20^{o}C for 50 minutes; (b) microwaves assisted extraction in 100 ml oil for 5 minutes; (c) maceration at 20^{o}C in 100 ml oil for 7 days. In this experiment were used the following oils: extra virgin sunflower oil, unrefined corn oil and refined rape seeds oil.

In the third experiment, dried and milled waste samples were extracted in extra virgin sunflower oil, unrefined corn oil and refine rape seeds oil at different waste concentration (2.5; 5; 10 and 20% weight/volume) and subjected to ultrasound at 20^{o}C for 50 minutes. Every experiment was performed in triplicate.

Every extraction were followed by filtering through filter paper Whatman No 1 and the resulted oil was collected in black plastic containers with threaded cap completely filled and store at 4^{o}C until they were analyzed. All oils were acquired from shops and some of them were used as control for the tests.

For both control and tested oils was determined total content of carotenoids using HPLC, peroxide index after UV accelerated oxidation, scavenger activity using DPPH method, oxidative susceptibility using Rancimat method and color based on CIELab parameters. Also, oils were subjected to thermal analysis by registering simultaneously mass variation (TG) and the coefficient of this variation (DTG) and also the difference between sample heat flow and

heat flow of the reference material. Thermoanalytical curves were registered as a function of temperature. Thermal stability was measured from starting temperature extrapolated of the first step of thermal decomposition from the respective TG curves. Combustion heat was estimated as the total area of the exothermic DSC peaks of the thermal decomposition that occurs during analysis of every oil.

6.3. Results and discussions

6.3.1. Carotenoids extraction from dried tomato by-products

Increase of carotenoids content after extraction of 5% dried tomatoes waste was ranged between 29.2 mg/kg and 38.0 mg/kg, reflecting carotenoids solubility in these oils (Table 6.1). Highest solubility was recorded in the extra virgin olive oil, followed by rice oil. Even though are having a similar fatty acids composition, it was recorded a significant difference between carotenoids solubility in extra virgin olive oil and pomace olive oil, probably due to the influence of the other compounds present in these oils. Benakmoum et al. (2008) [48] reported that carotenoids solubility in oils is increasing with the decrease of the chain of fatty acids of triglycerides while the degree of unsaturation of fatty acids had no influence upon carotenoids solubility.

Carotenoids content in oils increased significantly with the increase of the quantity of the dried tomato waste that was incorporated. Furthermore, it was discovered a linear dependence between the carotenoids content in enriched oil and the quantity of the tomato waste extracted, with determination coefficients R^2 of 0.995, 0.989 and 0.999 for extra virgin sunflower oil, unrefined corn oil and refined rape seeds oil respectively.

Extraction method significantly influenced the extracted quantity of carotenoids in oils (Table 6.2). For those three oils, the highest quantity was extracted using 7 days maceration, followed by ultrasounds assisted extraction and microwaves assisted extraction. However, UAE and MAE have lower time duration and assure a good extraction at a lower cost.

6.3.2. Influence of the extracted carotenoids from dried tomato waste on the oxidative stability of the oils

Carotenoids extraction from dried tomato waste induced a significant but contradictory change in the peroxide index values. While for the refined rape seeds oil, extra virgin olive oil, soy beans oil and grape seeds oil was determined a significant increase of respective peroxide index values after UAE of 5% tomato waste, for extra virgin sunflower oil, unrefined corn oil, refined sunflower oil and peanuts oil was determined a decrease of the respective peroxide index values. According some previous studies these contradictory changes in peroxide index values could be attributed to the antioxidant or pro-oxidant behavior of the carotenoids that can switch in certain conditions. Carotenoids can increase the formation of hydroperoxide for the triglycerides that suffer autoxidation, this pro-oxidant activity of carotenoids being connected to the oxygen concentration, carotenoids chemical structure and the presence of other antioxidants such as polyphenols and tocopherols [49, 50], compounds that are extracted together with the carotenoids from the tomatoes waste.

Extra virgin sunflower oil, unrefined corn oil and refined rape seeds oil enriched with carotenoids extracted by UAE or maceration of 5% dried tomatoes waste shown lower peroxide index values than those of the control oils.

A much higher value of peroxide index (2 to 8.7 folds higher) was determined in oils enriched with carotenoids using MAE of the dried tomatoes waste. These were expected results because it is well known that microwave heating accelerates lipids oxidation. Highest values of the peroxide index was registered for extra virgin sunflower oil enriched with carotenoids using a MAE method, in accordance with the highest level of PUFA found in this oil compared to the other oils that were tested. For most of the oils the extraction from dried tomatoes waste led to a slight but yet significant decrease of scavenger activity thus suggesting again that the extracted compounds could have a pro-oxidant activity.

6.3.3. The influence of carotenoids extracted from dried tomatoes waste upon the induction period of the oils

Table 6.1 shows the modification of the induction period of the oils after the extraction of dried tomatoes waste. Olive oils shown a longer induction period in accordance to the results reported by previous studies due to a point to the presence of tocopherol and phenolic compounds [51]. Determination of the induction period showed that carotenoids extraction from dried tomatoes waste increases the stability of certain oils (unrefined corn oil, refine sunflower oil and peanuts oil). In these cases, induction periods of the extraction oils seem to be significantly higher than those of the control oils. However, to the majority of the tested oils stability is decreasing after carotenoids extraction from dried tomatoes waste or there were not recorded significant differences between the induction period of the control oils and those of carotenoids enriched oils.

6.3.4. Thermal analysis

Figure 6.3 shows TG, DTG and DSC curves of extra virgin sunflower oil while Figure 6.4 presents thermo-analytical curves of extra virgin sunflower oils enriched with carotenoids extracted from dried tomatoes waste.

Comparative analysis of the thermogravimetric curves shows that sunflower oil was thermally stable until 206^{o}C while the carotenoid enriched oil after the extraction from dried tomatoes waste was thermally stable only until 177^{o}C. Until 250^{o}C the carotenoid enriched sunflower oil lost 7% of its initial mass while the control oil lost only 1.4% of its mass (figure 6.5). First step was exothermic loss of volatile compounds with low molecular mass, for the control sunflower oil the devolatilisation started at 182.0^{o}C while for the enriched oil this process started at 165.0^{o}C.

Liberated heat energy in every decomposing/combustion stage depends on oil. Thus, in devolatilisation stage, enthalpy variation was ΔH=-3214 J/g for sunflower oil and ΔH=-5723 J/g for carotenoids enriched sunflower oil. For both oils the devolatilisation process presented two peaks corresponding to the two major decomposing products at 325.6^{o}C and 353.0^{o}C for control sunflower oil and at 277.7^{o}C and 340.0^{o}C for carotenoids enriched oil.

Table 6.1. Total carotenoids content, free radical scavenger activity, Rancimat protection factor, peroxide index and CIELab parameters of the oils before and after UAE of 5% tomatoes waste*

	Total carotenoids content (mg/kg)	Scavenger activity	Protection factor (Rancimat)	Peroxide index (meq/kg)	L*	a*	b*
Extra virgin sunflower oil	1.6 ± 0.1^{a}	9.3 ± 0.3^{b}	-	183.0 ± 11.3^{b}	74.9 ± 0.2^{a}	0.0 ± 0.0^{a}	14.9 ± 0.2^{b}
Extra virgin sunflower oil, 5% tomatoes waste	34.8 ± 1.6^{b}	7.0 ± 0.2^{a}	0.94 ± 0.1	31.3 ± 1.6^{a}	77.3 ± 0.4^{b}	0.8 ± 0.0^{b}	5.5 ± 0.3^{a}
Unrefined corn oil	6.1 ± 0.4^{a}	6.5 ± 0.3^{b}	-	51.5 ± 2.8^{b}	73.7 ± 0.3^{a}	5.5 ± 0.1^{a}	72.2 ± 0.5^{b}
Unrefined corn oil, 5% tomatoes waste	38.4 ± 1.8^{b}	5.3 ± 0.2^{a}	1.25 ± 0.0	33.50 ± 1.8^{a}	76.1 ± 0.8^{b}	9.4 ± 0.4^{b}	69.8 ± 1.2^{a}
Refined rape seeds oil	nd^{a}	1.4 ± 0.1^{a}	-	33.7 ± 1.1^{a}	76.9 ± 0.2^{a}	0.0 ± 0.0^{a}	3.9 ± 0.2^{b}
Refined rape seeds oil, 5% tomatoes waste	35.4 ± 0.9^{b}	1.7 ± 0.1^{b}	0.88 ± 0.0	163.8 ± 9.6^{b}	78.0 ± 0.6^{b}	0.8 ± 0.0^{b}	1.6 ± 0.1^{a}
Extra virgin olive oil	2.1 ± 0.1^{a}	18.9 ± 0.8^{b}	-	26.5 ± 1.5^{a}	72.2 ± 0.3^{a}	7.8 ± 0.1^{b}	58.8 ± 0.9^{b}
Extra virgin olive oil, 5% tomatoes waste	40.1 ± 2.2^{b}	9.4 ± 0.5^{a}	0.82 ± 0.0	78.0 ± 3.5^{b}	73.7 ± 0.8^{b}	5.5 ± 0.3^{a}	30.6 ± 1.1^{a}
Pomace olive oil	nd^{a}	1.2 ± 0.1^{a}	-	6.9 ± 2.9^{a}	76.1 ± 0.2^{a}	1.6 ± 0.1^{a}	7.8 ± 0.2^{b}
Pomace olive oil, 5% tomatoes waste	29.2 ± 1.3^{b}	1.0 ± 0.1^{a}	0.87 ± 0.0	14.0 ± 5.9^{b}	76.1 ± 0.6^{a}	2.4 ± 0.2^{b}	5.5 ± 0.4^{a}
Soy beans oil	3.3 ± 0.3^{a}	1.1 ± 0.1^{b}	-	38.6 ± 1.8^{a}	76.1 ± 0.2^{a}	0.0 ± 0.2^{a}	8.6 ± 0.1^{b}
Soy beans oil, 5% tomatoes waste	39.8 ± 1.8^{b}	0.6 ± 0.0^{a}	1.01 ± 0.1	258.4 ± 11.6^{b}	78.0 ± 0.4^{b}	0.6 ± 0.2^{b}	0.1 ± 0.1^{a}
Refined sunflower oil	nd^{a}	0.8 ± 0.0^{a}	-	96.1 ± 5.1^{b}	75.7 ± 0.1^{a}	0.1 ± 0.1^{a}	6.3 ± 0.1^{b}
Refined sunflower oil, 5% tomatoes waste	34.8 ± 1.5^{b}	0.7 ± 0.1^{a}	1.19 ± 0.0	35.9 ± 1.9^{a}	78.8 ± 0.3^{b}	0.9 ± 0.1^{b}	0.0 ± 0.1^{a}
Peanuts oil	nd^{a}	0.5 ± 0.0^{a}	-	28.6 ± 16.3^{b}	73.3 ± 0.2^{a}	0.1 ± 0.0^{a}	25.1 ± 0.2^{b}
Peanuts oil, 5% tomatoes waste	36.4 ± 1.4^{b}	1.1 ± 0.1^{b}	3.15 ± 0.1	3.9 ± 0.2^{a}	78.4 ± 0.5^{b}	0.6 ± 0.1^{b}	0.8 ± 0.1^{a}
Rice oil	nd^{a}	0.3 ± 0.0^{a}	-	5.9 ± 0.4^{a}	75.3 ± 0.2^{a}	0.0 ± 0.1^{a}	11.8 ± 0.1^{b}
Rice oil, 5% tomatoes waste	37.6 ± 2.1^{b}	0.3 ± 0.0^{a}	1.17 ± 0.1	16.3 ± 0.9^{b}	78.0 ± 0.6^{b}	0.8 ± 0.1^{b}	1.6 ± 0.1^{a}
Grape seeds oil	16.7 ± 0.5^{a}	0.5 ± 0.0^{b}	-	17.8 ± 0.7^{a}	75.3 ± 0.3^{a}	0.0 ± 0.1^{a}	11.0 ± 0.2^{b}
Grape seeds oil, 5% tomatoes waste	50.6 ± 2.8^{b}	0.1 ± 0.0^{a}	0.91 ± 0.1	151.7 ± 7.7^{b}	77.3 ± 0.5^{b}	0.8 ± 0.1^{b}	0.8 ± 0.1^{a}

* Data with different exponent letters are significantly different ($p<0.05$)

Table 6.2. Total carotenoids content, scavenger activity, Rancimat protection factor, peroxide index and CIELab parameters of the oils before and after UAE of 5% tomatoes waste: influence of the extraction method*

	Total carotenoids content (mg/kg)	Scavenger activity	Protection factor (Rancimat)	Peroxide index (meq/kg)	L*	a*	b*
Extra virgin sunflower oil							
Control	1.6 ± 0.1^{a}	9.3 ± 0.3^{d}	-	183.00 ± 11.3^{b}	74.9 ± 0.2^{a}	0 ± 0.0^{a}	14.9 ± 0.2^{b}
Microwaves assisted extraction	32.2 ± 1.3^{b}	8.2 ± 0.3^{c}	0.88 ± 0.1^{a}	363.90 ± 16.5^{c}	75.7 ± 0.6^{ab}	1.6 ± 0.1^{c}	22.0 ± 0.6^{d}
Ultrasounds assisted extraction	34.8 ± 1.6^{c}	7.0 ± 0.2^{b}	0.94 ± 0.1^{a}	31.30 ± 1.6^{a}	77.3 ± 0.4^{c}	0.8 ± 0.0^{b}	5.5 ± 0.3^{a}
Maceration for 7 days	40.2 ± 1.8^{d}	5.3 ± 0.2^{a}	0.97 ± 0.1^{a}	32.20 ± 1.8^{a}	76.5 ± 0.8^{bc}	0.8 ± 0.1^{b}	16.5 ± 0.4^{c}
Unrefined corn oil							
Control	6.1 ± 0.4^{a}	6.5 ± 0.3^{b}	-	51.50 ± 2.8^{b}	73.7 ± 0.3^{a}	5.5 ± 0.1^{a}	72.2 ± 0.5^{c}
Microwaves assisted extraction	35.2 ± 1.6^{b}	5.2 ± 0.3^{a}	1.35 ± 0.1^{b}	215.60 ± 12.3^{c}	76.1 ± 0.5^{b}	7.8 ± 0.2^{b}	54.9 ± 0.6^{a}
Ultrasounds assisted extraction	38.4 ± 1.8^{c}	5.3 ± 0.2^{a}	1.25 ± 0.0^{a}	33.50 ± 1.8^{a}	76.1 ± 0.8^{b}	9.4 ± 0.4^{c}	69.8 ± 1.2^{b}
Maceration for 7 days	40.9 ± 1.5^{c}	5.0 ± 0.3^{a}	1.43 ± 0.1^{b}	36.70 ± 1.8^{a}	76.5 ± 0.5^{b}	7.8 ± 0.3^{b}	73.7 ± 0.8^{c}
Refined rape seeds oil							
Control	nd^{a}	1.4 ± 0.1^{a}	-	33.70 ± 1.1^{a}	76.9 ± 0.2^{a}	0.0 ± 0.0^{a}	3.9 ± 0.2^{b}
Microwaves assisted extraction	32.3 ± 1.1^{b}	1.4 ± 0.2^{a}	0.88 ± 0.1^{a}	294.50 ± 15.9^{c}	77.6 ± 0.4^{ab}	0.4 ± 0.1^{b}	18.0 ± 0.9^{d}
Ultrasounds assisted extraction	35.4 ± 0.9^{c}	1.7 ± 0.1^{b}	0.88 ± 0.0^{a}	63.80 ± 3.6^{b}	78.0 ± 0.6^{b}	0.8 ± 0.0^{c}	1.6 ± 0.1^{a}
Maceration for 7 days	37.6 ± 1.7^{d}	1.4 ± 0.1^{a}	0.87 ± 0.0^{a}	25.30 ± 1.1^{a}	77.6 ± 0.8^{ab}	0.8 ± 0.1^{c}	8.6 ± 0.5^{c}

* Data with different exponent letters are significantly different ($p<0.05$)

Table 6.3. Total carotenoids content, scavenger activity, Rancimat protection factor, peroxide index and CIELab parameters of the oils before and after UAE of 5% tomatoes waste*:* influence of the tomatoes waste concentration*

Concentration of the tomatoes waste	Total carotenoids content (mg/kg)	Scavenger activity	Protection factor (Rancimat)	Peroxide index (meq/kg)	L*	a*	b*
Extra virgin sunflower oil							
Control	1.6 ± 0.1^{a}	9.3 ± 0.3^{e}	-	183.0 ± 11.3^{c}	74.9 ± 0.2^{ab}	0.0 ± 0.0^{a}	14.9 ± 0.2^{b}
2.5%	25.7 ± 0.8^{b}	5.3 ± 0.3^{c}	1.02 ± 0.1^{a}	124.3 ± 5.8^{b}	77.6 ± 0.4^{c}	0.0 ± 0.1^{a}	9.4 ± 0.4^{a}
5%	34.8 ± 1.6^{c}	7.0 ± 0.2^{d}	0.94 ± 0.1^{a}	31.3 ± 1.6^{a}	77.3 ± 0.4^{c}	0.8 ± 0.0^{b}	15.5 ± 0.3^{b}
10%	85.4 ± 3.6^{d}	2.5 ± 0.1^{b}	1.01 ± 0.1^{a}	232.1 ± 10.5^{d}	75.3 ± 0.8^{b}	0.8 ± 0.0^{b}	21.2 ± 0.8^{c}
20%	168.7 ± 6.6^{e}	2.1 ± 0.1^{a}	0.97 ± 0.0^{a}	250.2 ± 12.1^{e}	74.1 ± 0.8^{a}	0.8 ± 0.1^{b}	33.7 ± 1.4^{d}
Unrefined corn oil							
Martor	6.1 ± 0.4^{a}	6.5 ± 0.3^{c}	-	51.5 ± 2.8^{b}	73.7 ± 0.3^{a}	5.5 ± 0.1^{b}	72.2 ± 0.5^{ab}
2.5%	8.2 ± 0.5^{a}	4.9 ± 0.3^{a}	1.55 ± 0.1^{a}	39.0 ± 1.9^{a}	76.5 ± 0.6^{c}	7.8 ± 0.2^{c}	73.7 ± 2.0^{b}
5%	38.4 ± 1.8^{b}	5.3 ± 0.2^{ab}	1.25 ± 0.0^{a}	215.6 ± 12.3^{c}	76.1 ± 0.8^{bc}	9.4 ± 0.4^{d}	69.8 ± 1.2^{a}
10%	84.1 ± 4.8^{c}	5.5 ± 0.2^{b}	1.21 ± 0.1^{a}	37.3 ± 1.5^{a}	75.3 ± 0.2^{b}	5.5 ± 0.1^{b}	72.9 ± 1.8^{b}
20%	162.7 ± 7.5^{d}	5.3 ± 0.3^{ab}	1.41 ± 0.1^{a}	31.5 ± 0.9^{a}	72.9 ± 0.5^{a}	0.0 ± 0.2^{a}	72.2 ± 1.6^{ab}
Refined rape seeds oil							
Martor	nd^{a}	1.4 ± 0.1^{d}	-	33.7 ± 1.1^{a}	76.9 ± 0.2^{b}	0.0 ± 0.0^{a}	3.9 ± 0.2^{a}
2.5%	18.2 ± 0.8^{b}	0.8 ± 0.1^{b}	0.96 ± 0.0^{ab}	32.7 ± 1.4^{a}	77.6 ± 0.2^{bc}	0.8 ± 0.1^{b}	8.6 ± 0.5^{b}
5%	35.4 ± 0.9^{c}	1.7 ± 0.1^{e}	0.88 ± 0.0^{a}	163.8 ± 9.6^{b}	78.0 ± 0.6^{c}	0.8 ± 0.0^{b}	11.6 ± 0.1^{c}
10%	75.3 ± 2.8^{d}	0.6 ± 0.1^{a}	0.98 ± 0.1^{ab}	32.5 ± 1.2^{a}	74.5 ± 0.5^{a}	0.8 ± 0.1^{b}	25.9 ± 1.4^{d}
20%	142.8 ± 6.9^{e}	1.2 ± 0.1^{c}	1.04 ± 0.0^{b}	36.3 ± 1.6^{a}	74.1 ± 0.6^{a}	0.8 ± 0.1^{b}	38.4 ± 1.7^{e}

* Data with different exponent letters are significantly different ($p<0.05$)

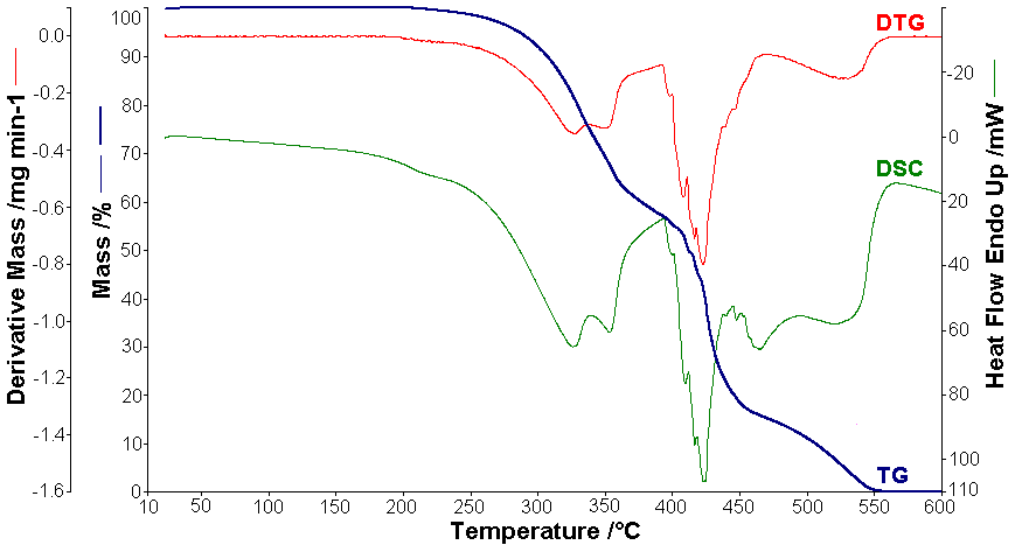

Figure 6.3. Thermoanalytical curves (TG, DTG și DSC) of the sunflower oil control sample.

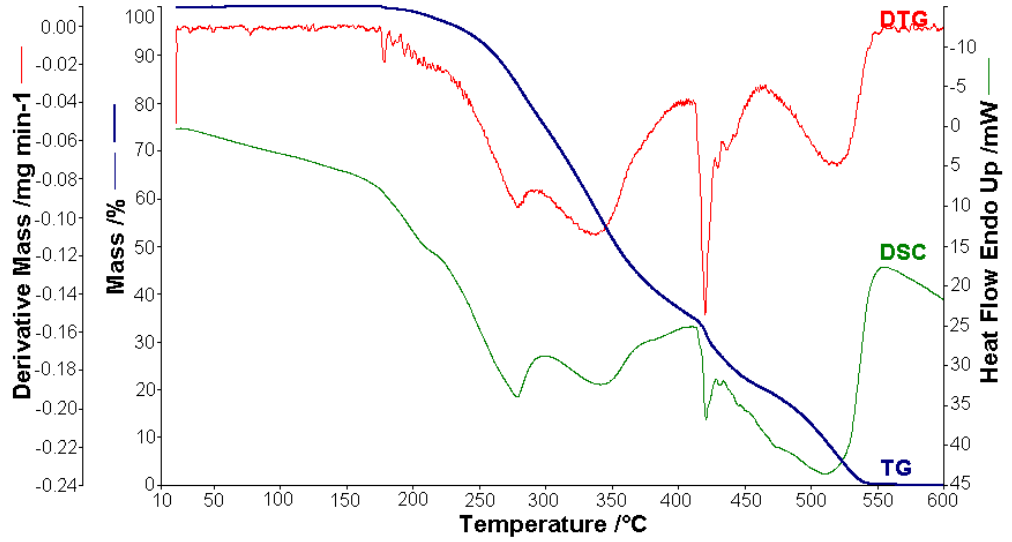

Figure 6.4. Thermoanalytical curves (TG, DTG and DSC) of the sunflower oil enriched with carotenoids after extraction from dried tomatoes waste.

As well, were registered differences also in the decomposition phase of the resinification compounds (which occurs after the first stage of decomposition), when the exothermic effect are ΔH = - 6153 J/g for sunflower oil and ΔH = - 5937 J/g for carotenoid enriched sunflower oil, even though peaks are reached at similar values of temperature (423^{o}C and 421^{o}C respectively).

Extraction into oil of tomatoes waste compounds lead to a decrease of the temperature of oxidation start from 263.72^{o}C to 221.04^{o}C.

These results are well correlated to those obtained using the Rancimat method, suggesting once again that those compounds that were extracted from the tomatoes waste, including carotenoids, exert a pro-oxidant effect and induce a decrease of oxidative stability of the extra virgin sunflower oil.

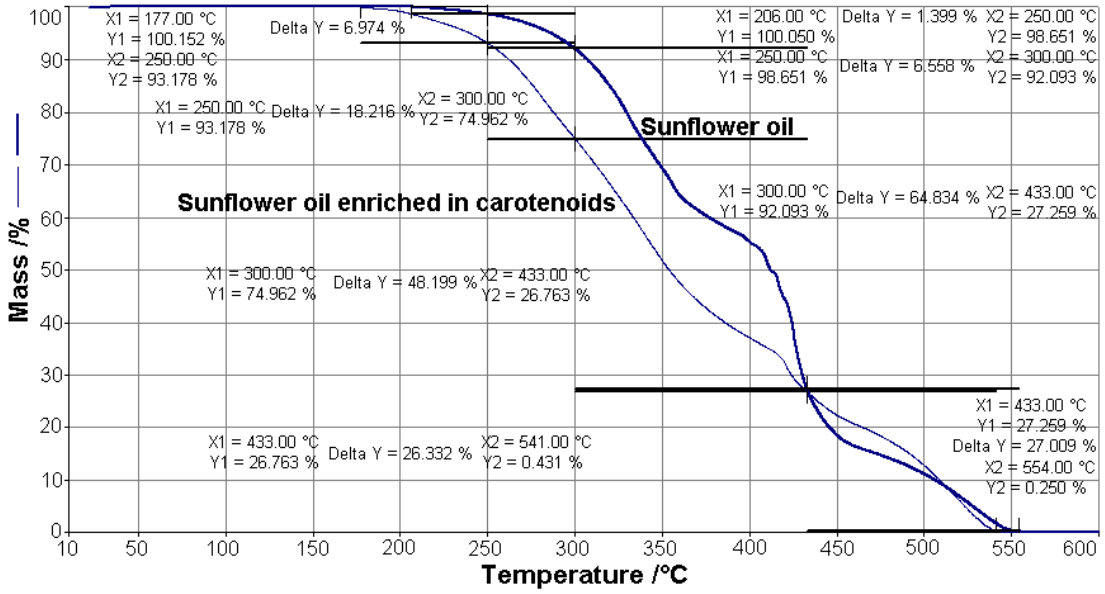

Figure 6.5. TG curves of sunflower oils: control and enriched with carotenoids after extraction from dried tomatoes waste.

6.3.4. Oils color change after extraction

Tables 6.2 - 6.3 show the levels of CIELab parameters (L*, a*, b*) for the oils obtained after each experiment.

Extraction from dried tomatoes waste is influencing significantly the parameters that define color of the oils ($p<0.05$). Luminosity (L*) varied from 72.2 to 78.8 in this present study. L* values increase with the increase of the dried tomatoes waste concentration until 5% and after that they decreased, suggesting that extraction from a higher concentration is leading to a blackening of the oils. Values a* increased while values b* decreased after extraction and these changes can be attributed to the carotenoids extraction from waste into oil, prevailing red lycopene.

6.4. Partial conclusions

Extraction from dried tomatoes waste improved thermal and oxidative stability for some of the oils, while for others produced an increase of peroxide index and a decrease of induction time as it was determined using Rancimat method. In most of the oil extraction caused a slight but yet significant decrease of scavenger activity.

For the extra virgin sunflower oil, extraction from dried tomatoes waste produced a decrease of the temperature at which oxidation is starting from 263.72°C to 221.04°C as it was determined using differential scanning calorimetry. For these oils results obtained using Rancimat and DSC methods seem to be consistent, showing pro-oxidant effects of the compounds extracted from dried tomatoes waste. Extraction from dried tomatoes waste into vegetable oil can lead to obtain a colored oil that can be used in food industry without the additional usage of a potential harmful organic solvents. This oil can be a potential source of bioactive compounds and can have a significant antioxidant activity when is ingested as part of a food diet.

CHAPTER 7

ENRICHING OILS WITH CAROTENOIDS EXTRACTED FROM SECUNDARY PRODUCTS RESULTED AFTER PROCESSING SEA BUCKTHORN FRUITS (*Hippophae rhamnoides* ssp. *sinensis*)

7.1. Study opportunity

In this study the secondary products resulted after processing sea buckthorn fruits were used as a source of natural carotenoids for enriching vegetable oils. For extracting carotenoids directly in vegetable oils have been used maceration and UAE. In the dried secondary product was evaluated carotenoids total content, carotenoids profile and antioxidant activity. Resulted oils were analyzed in order to determine total carotenoid content, antioxidant activity ABTS and color. Oxidative stability of the oil was determined using peroxide index method; while thermal stability was determined through thermogravimetric analyze (TGA) and differential scanning calorimetry (DSC).

7.2. Materials and methods

Secondary products resulted after processing sea buckthorn fruits were obtaine from Biocat Prod S.R.L. a producer and industrial processing of sea buckthorn fruits, from Gr[dina, county of Constanța, in south-east of Rmania (44°33'N, 28°26'E). Secondary products were dehydrated in an industrial drier (Blue Spark Systems SR.L., Romania) at 60^{O}C, and then were milled. Samples of dried and milled sea buckthorn waste (SBP) were undergoing ultrasound assisted extraction and maceration in extra virgin sunflower oil (EVS), extra virgin olive oil (EVO) and refined sunflower oil (RS) in different concentrations (2.5; 5 and 10%). For all oils, both enriched and control, the carotenoids total content, peroxide index, antioxidant activity ABTS and CIELab parameters were determined. Oils thermal behavior was analyzed using a thermal analyzer DIAMOND TG / DTA, in dynamic air atmosphere (150 cm^3/min) in a non-isometric linear regimen with a heating constant rate of 10 k/min.

Were simultaneously registered mass variations determined through thermogravimetric analyze (TG curve), derivate thermogravimetric analyze (DTG curve) and the difference in heat exchange between sample and the reference material (DSC). Thermal stability was measured from starting temperature extrapolated of the first step of thermal decomposition from the respective TG curves.

7.3. Results and discussions

7.3.1. Carotenoids content in secondary products resulted from sea buckthorn processing

Total carotenoids content in secondary products resulted from sea buckthorn processing was 24.56 ± 1.55 mg/100 g, and it was determined using a spectrophotometric method while total carotenoids content determined with HPLC was only 16.67 mg/100 g.

Zeaxanthin and β-carotene were the main carotenoids identified in sea buckthorn secondary products (6.96 mg/100 g and 8.7 mg/100 g respectively).

7.3.2. Total carotenoids content in oils

Between all three oils, EVO had the biggest carotenoid content (21.5 mg/kg), followed by EVS (6.3 mg/kg) and RS (4.8 mg/kg) (table 7.2). After 50 minutes of UAE of 10% SBP total carotenoids content varied from 137.83 mg/kg (RS) and 157.48 mg/kg (EVO). UAE recovered a significant higher quantity of carotenoids from SBP compared to maceration. Total carotenoids content in EVO was 29% higher after 50 minutes of UAE than after 10 days of maceration, while in sunflower oils was registered an increase between 9.5% and 33.3%.

7.3.3. Antioxidant activity

EVO presented the highest scavenging activity of 5.55 mmol Trolox/kg compared to 4.47 mmol Trolox/kg and 4.16 mmol Trolox/kg for EVS and RS respectively (Table 7.2). Enrichment with carotenoids determined an increase of ABTS antioxidant activity and the most significant was recorded after the extraction of SBP.

7.3.4. Peroxide index

After UV irradiation the highest peroxide value was found for EVS while the lowest was found for EVO. Though EVS is rich in tocopherols it is almost free of phenolic compounds, while EVO contains both tocopherols and phenolic compounds as antioxidants [17].

SBP extraction in oil led to a small decrease of peroxide index but the differences were significant ($P<0.05$) only for the extraction of 10% SBP. RS was more stable against UV accelerated oxidation compared to EVS. In EVS, SBP extraction determined in general an increase of peroxide index but the differences were not significant. However, RS enrichment with carotenoids extracted from SBP determined a significant increase of peroxide index.

Previous studies suggested that these contradictory changes of peroxide index occur due to the carotenoids pro-oxidant or anti-oxidant behavior that can manifest in certain conditions.

7.3.5. Thermal analysis and calorimetric study

Thermal stability and behavior at thermal degradation of carotenoids enriched oils, after the extraction of 5% SBP (RS, EVS, EVO) were investigate using a non-linear heating program in controlled atmosphere. For comparative purposes, in the exactly same experimental conditions were thermally tested also the control oils, and SBP alone. In Figure 7.2 are presented TG, DT and DSC curves for RS (called control) while the curves for carotenoids enriched RS oil after extraction of SBP are presented in Figure 7.3. For a better understanding of the influence manifested by carotenoids on the stability of edible oils was also performed a thermal analysis for SBP (Figure 7.4).

By extracting carotenoids from SBP in RS thermokinetic stability of the system is diminished (Figure 7.6).

Table 7.2. Total carotenoids content, peroxide index and ABTS antioxidant activity of control and carotenoids enriched oils after extraction from SBP

	Total carotenoids content (mg/kg)		Peroxide index (meq/kg)		ABTS antioxidant activity (mmol Trolox/kg)	
	UAE	Maceration	UAE	Maceration	UAE	Maceration
EVO	21.50 ± 0.89^{A}	21.50 ± 0.89^{A}	45.50 ± 1.66^{BC}	45.50 ± 1.66^{B}	5.55 ± 0.21^{A}	5.55 ± 0.21^{A}
EVO+2.5%SBP	$76.91 \pm 1.82_{b}^{B}$	$56.57 \pm 2.77_{a}^{B}$	$46.44 \pm 1.78_{a}^{C}$	$51.43 \pm 2.31_{b}^{C}$	$5.37 \pm 0.30_{a}^{A}$	$5.73 \pm 0.19_{a}^{A}$
EVO+5.0%SBP	$98.84 \pm 3.09_{b}^{C}$	$73.69 \pm 2.78_{a}^{C}$	$43.65 \pm 0.98_{a}^{B}$	$42.14 \pm 1.88_{a}^{B}$	$5.27 \pm 0.15_{a}^{A}$	$6.75 \pm 0.26_{b}^{B}$
EVO+10.0%SBP	$157.84 \pm 6.67_{b}^{D}$	$122.28 \pm 4.33_{a}^{D}$	$35.18 \pm 1.21_{a}^{A}$	$37.52 \pm 1.26_{a}^{A}$	$5.35 \pm 0.34_{a}^{A}$	$6.90 \pm 0.22_{b}^{B}$
EVS	6.30 ± 0.28^{A}	6.30 ± 0.28^{A}	153.53 ± 6.44^{A}	153.47 ± 6.44^{A}	4.47 ± 0.20^{A}	4.47 ± 0.20^{A}
EVS+2.5%SBP	$40.77 \pm 1.94_{a}^{B}$	$45.49 \pm 1.42_{b}^{B}$	$159.11 \pm 5.81_{a}^{AB}$	$162.56 \pm 6.66_{a}^{A}$	$4.55 \pm 0.17_{a}^{A}$	$4.72 \pm 0.14_{a}^{A}$
EVS+5.0%SBP	$74.79 \pm 3.38_{b}^{C}$	$56.11 \pm 2.06_{a}^{C}$	$166.21 \pm 6.77_{a}^{B}$	$161.62 \pm 5.09_{a}^{A}$	$4.83 \pm 0.22_{a}^{AB}$	$4.74 \pm 0.18_{a}^{A}$
EVS+10.0%SBP	$143.93 \pm 5.56_{b}^{D}$	$121.28 \pm 5.51_{a}^{D}$	$161.58 \pm 4.89_{a}^{AB}$	$159.39 \pm 5.79_{a}^{A}$	$5.12 \pm 0.24_{a}^{B}$	$4.77 \pm 0.21_{a}^{A}$
RS	4.80 ± 0.21^{A}	4.80 ± 0.21^{A}	116.82 ± 3.66^{A}	116.82 ± 3.66^{A}	$4.16 \pm 0.09_{a}^{A}$	$4.16 \pm 0.09_{a}^{A}$
RS+2.5%SBP	$43.41 \pm 1.65_{b}^{B}$	$38.17 \pm 1.56_{a}^{B}$	$112.86 \pm 4.08_{a}^{A}$	$141.54 \pm 5.56_{b}^{C}$	$4.21 \pm 0.16_{a}^{A}$	$4.33 \pm 0.19_{a}^{AB}$
RS+5.0%SBP	$82.65 \pm 3.35_{b}^{C}$	$64.75 \pm 2.93_{a}^{C}$	$121.65 \pm 5.77_{a}^{A}$	$128.52 \pm 3.68_{a}^{B}$	$4.28 \pm 0.19_{a}^{A}$	$4.56 \pm 0.15_{a}^{BC}$
RS+10.0%SBP	$137.83 \pm 6.65_{b}^{D}$	$120.34 \pm 5.13_{a}^{D}$	$141.08 \pm 6.14_{a}^{B}$	$143.45 \pm 4.87_{a}^{C}$	$4.59 \pm 0.17_{a}^{B}$	$4.63 \pm 0.14_{a}^{C}$

*Value from the same column for the same type of oil that having different capital letters as exponent are significantly different $P<0.05$;

**Values for the same row for the same characteristic followed by small letters as index are significantly different $P<0.05$.

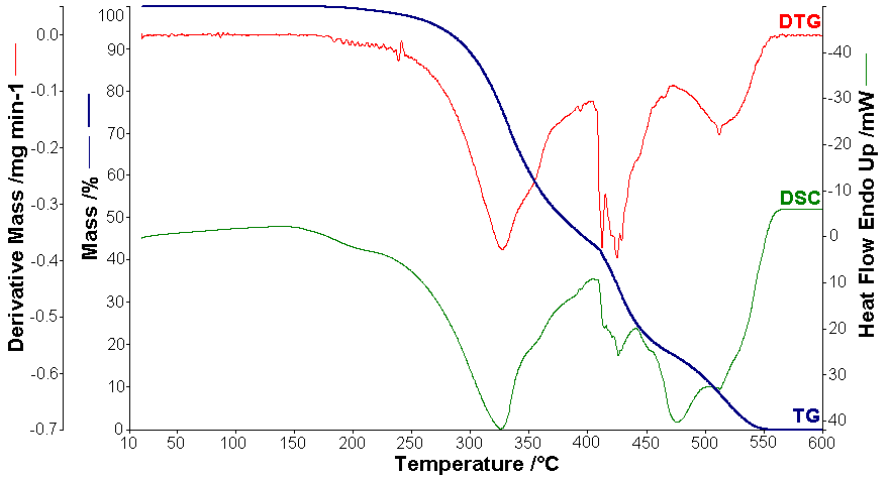

Figure 7.2. Thermoanalytical curves of RS (control) in air dynamic atmosphere at 10 K/min.

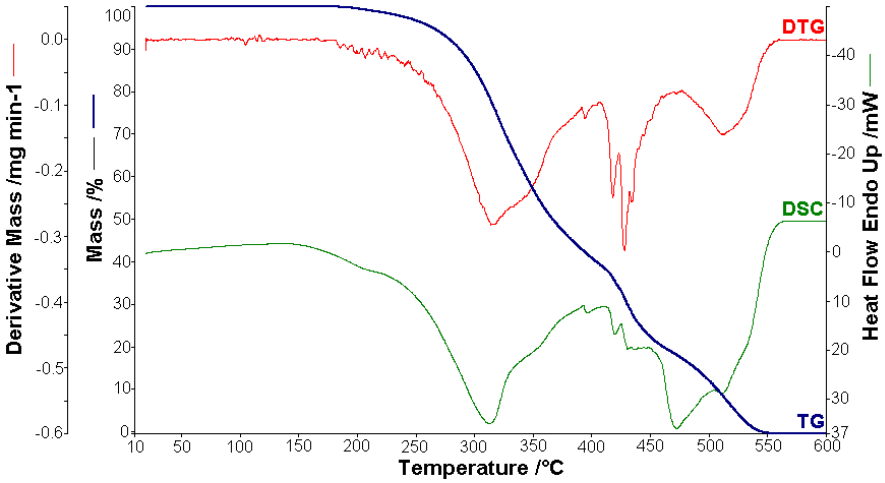

Figure 7.3. Thermoanalytical curves of RS carotenoids enriched after direct extraction from SBP, in air dynamic atmosphere at 10 K/min.

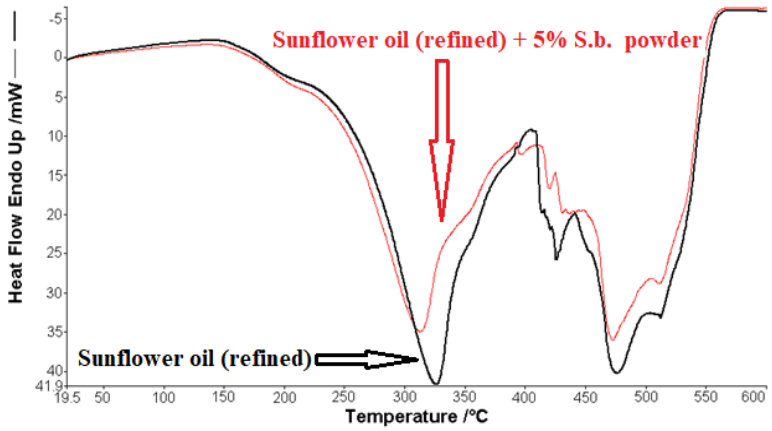

Figure 7.6. DSC curves of RS (control) against RS carotenoids enriched after SBP direct extraction in air dynamic atmosphere at 10 K/min.

Carotenoid compounds extraction from SBP in oils determined a very small decrease, approximately 4-5^{o}C, of the oxidation starting temperature and a decrease of DSC peak temperature of approximately 11-12^{o}C (Table 7.3). These values are lower than those registered as the effect of carotenoids extraction from dried tomato waste in a previous study [52]. These results are in correlation with those obtained previously [52]. Results shown the low pro-oxidant effect of carotenoid compounds extracted, these compounds were not determining a significant decrease of the thermal and oxidative stability of the analyzed oils.

Table 7.3. Thermal parameters of oxidation start and DSC peak

Tipul uleiului	Control		5% Carotenoid-enriched (SBP) oils	
	DSC oxidation onset temperature/°C	DSC oxidation peak temperature /°C	DSC oxidation onset temperature/°C	DSC oxidation peak temperature /°C
RS	153	325	148	312
EVS	176	344	172	334
EVO	189	314	177	303

7.3.6. Color

In extra virgin olive oil, the extraction of SBP determined the increase of lightness (L*), while in sunflower oils, the L* values decreased; therefore, the extraction resulted in the darkening of these oils. The a* and b* values increased significantly with increase in levels of added SBP, indicating increase in redness and yellowness appearance, respectively. These changes could be attributed to the extraction of carotenoids, predominantly β-carotene and zeaxanthin, in the oils. Besides the nutritional aspects related to the increase of the oil functionality, the enrichment with carotenoids of the oils leads to the improvement of the chromatic characteristics of the oils which may increase consumer attractiveness and confidence.

7.4. Partial conclusion

Based on the results of this study, sea buckthorn by-products are a good source of carotenoids, mainly zeaxanthin and β-carotene. Extraction of these by-products in edible vegetable oils enhanced significantly the carotenoid content and contributed to the increase of radical scavenging activity of the oils. Ultrasonic-assisted extraction led to a significantly higher recovery of carotenoids from dried sea buckthorn by-products than maceration. The thermodynamic stability of the sunflower oils and olive oil is not greatly affected by the enrichment with carotenoids from sea buckthorn by-products, while only the thermokinetic stability may diminish; therefore, the thermal oxidative stability of the carotenoids-edible oils systems is fairly good, presumably the shelf life being the same as in the case of the initial edible oils, with only a high increase in temperature that may eventually lower it. One may conclude that the carotenoids from sea buckthorn by-products may be safely used for their coloring effect and for the appeal to enhance the acceptability and valorization of the edible oils in order to be further sold.

Tableul 7.4. CIELab parameters of the control oils and of the oils enriched in carotenoids after extraction of dried sea buckthorn (*Hippophae¨ rhamnoides*) by-products

	L^*		a^*		b^*	
	UAE	Maceration	UAE	Maceration	UAE	Maceration
EVO	78.76 ± 0.62^{A}	78.76 ± 0.62^{A}	-2.10 ± 0.12^{A}	-2.10 ± 0.12^{A}	38.29 ± 5.26^{A}	38.29 ± 5.26^{A}
EVO+2.5%SBP	$83.19 \pm 1.48_{a}^{B}$	$83.13 \pm 1.29_{a}^{B}$	$-0.76 \pm 0.39_{a}^{B}$	$-0.58 \pm 0.41_{a}^{A}$	$47.42 \pm 3.68_{a}^{B}$	$49.95 \pm 1.79_{a}^{B}$
EVO+5.0%SBP	$84.02 \pm 1.04_{b}^{B}$	$80.36 \pm 1.17_{a}^{AB}$	$0.10 \pm 0.47_{a}^{B}$	$1.67 \pm 0.46_{b}^{B}$	$58.14 \pm 3.03_{a}^{C}$	$61.39 \pm 3.93_{a}^{C}$
EVO+10.0%SBP	$80.92 \pm 1.34_{a}^{A}$	$79.61 \pm 2.43_{a}^{A}$	$4.10 \pm 1.27_{a}^{C}$	$4.52 \pm 1.78_{a}^{C}$	$70.08 \pm 2.23_{a}^{D}$	$66.87 \pm 5.03_{a}^{C}$
EVS	83.21 ± 0.61^{C}	83.21 ± 0.61^{B}	-0.77 ± 0.20^{A}	-0.77 ± 0.20^{A}	18.55 ± 2.18^{A}	18.55 ± 2.18^{A}
EVS+2.5%SBP	$78.48 \pm 0.80_{a}^{AB}$	$79.75 \pm 1.05_{a}^{A}$	$1.02 \pm 0.23_{a}^{AB}$	$0.43 \pm 0.59_{a}^{A}$	$49.38 \pm 4.20_{a}^{B}$	$48.85 \pm 1.86_{a}^{B}$
EVS+5.0%SBP	$80.08 \pm 1.57_{a}^{B}$	$78.90 \pm 0.83_{a}^{A}$	$2.64 \pm 1.16_{a}^{B}$	$2.78 \pm 0.58_{a}^{B}$	$58.38 \pm 5.91_{a}^{BC}$	$61.29 \pm 0.67_{a}^{C}$
EVS+10.0%SBP	$76.42 \pm 1.25_{a}^{A}$	$77.99 \pm 1.64_{a}^{A}$	$7.13 \pm 2.33_{a}^{C}$	$6.51 \pm 2.23_{a}^{C}$	$65.25 \pm 9.85_{a}^{C}$	$71.07 \pm 6.42_{a}^{D}$
RS	83.52 ± 1.52^{B}	83.52 ± 1.52^{AB}	-0.12 ± 0.15^{A}	-0.12 ± 0.15^{A}	9.21 ± 0.57^{A}	9.21 ± 0.57^{A}
RS+2.5%SBP	$82.67 \pm 0.51_{a}^{B}$	$83.82 \pm 1.35_{a}^{B}$	$-0.29 \pm 0.17_{a}^{A}$	$-0.36 \pm 0.28_{a}^{A}$	$46.90 \pm 1.05_{a}^{B}$	$42.75 \pm 4.61_{a}^{B}$
RS+5.0%SBP	$80.40 \pm 0.80_{a}^{A}$	$81.71 \pm 2.12_{a}^{AB}$	$2.63 \pm 0.67_{a}^{B}$	$1.81 \pm 1.38_{a}^{B}$	$61.94 \pm 4.83_{a}^{C}$	$55.39 \pm 8.68_{a}^{C}$
RS+10.0%SBP	$79.73 \pm 0.70_{a}^{A}$	$80.75 \pm 0.79_{a}^{A}$	$5.62 \pm 0.59_{a}^{C}$	$5.25 \pm 0.64_{a}^{C}$	$69.51 \pm 0.87_{a}^{D}$	$69.11 \pm 3.66_{a}^{D}$

*Values in the same column for the same type of oil followed by different superscript upper-case letters are significantly different at P<0.05
**Values in the same row for the same parameter followed by different subscript lower-case letters are significantly different at P<0.05

CHAPTER 8

DEVELEPMENT AND VALIDATION OF A REVERSE PHASE HPLC METHOD FOR THE ANALYSIS OF CAROTENOIDS IN EGG YOLK

8.1. Study opportunity

This study was focused on the development and validation of an adequate reliable, fast and simple HPLC method, using a column C18 and UV-Vis detection (DAD) for the simultaneous detection of egg yolk carotenoids.

8.2. Materials and methods

8.2.1. Reagents and standards

Standards of lutein, zeaxanthin, canthaxanthin, astaxanthin, lycopene, β-carotene and *trans*-β-apo-8'-carotenal were acquired from Sigma-Aldrich (Chemie, Steinheim, Germany). Eggs used for this study were collected from hens fed with a diet enriched with by-products from tomatoes processing (peels+seeds). HPLC analyze was performed using a Finningan Surveyor Plus system (Thermo Electron Corporation, San Jose, CA).

8.2.4. Carotenoids extraction

Carotenoids were extracted from 0.5 g sample with 10 mL of petroleum ether:methanol:ethyl acetate (1:1:1, v/v/v) containing 0.1% butyl hydroxytoluene (BHT) by homogenizing for 5 min at 2500 rpm using a Vortex homogenizer. The sample was centrifuged for 6 min at 6000 rpm and the supernatant was collected. The residue was extracted following the same procedure until the supernatant was colorless. The combined supernatants were washed by adding 10 mL of 5% NaCl solution, mixing vigorously and incubating for 30 min until two layers were separated. The upper layer was collected, evaporated to dryness under N2 flow and then re-dissolved in 2 mL of acetonitrile:methanol:ethyl acetate (60:20:20, v/v/v) containing butylated hydroxytoluene (BHT) (1% w/v). The final solution was filtered through 0.45 µm membrane filters for HPLC injection.

8.2.5. Chromatographic conditions

The mobile phase system comprised acetonitrile:methanol (95:5, v/v) (A), acetonitrile:methanol:ethyl acetate (60:20:20, v/v/v) (B) and water (C). Carotenoids were eluted at a flow rate of 1.5 mL/min with the following gradient: 96% A and 4% C in the beginning, maintained for 10 min, changed linearly to 100% B in 13 min, maintained 5 min and returned to 96% A and 4% C in 2 min. Quantification was performed using Chrom Quest 4.2 software by comparing peak area with standard reference curves.

8.2.6. Method validation

The linearity of the answer of the detector was tested by preparing five mixt solutions for calibration. Calibration curves were established by graphic representation of the areas as a function of analytes concentration. Correlation coefficients were used for measuring linearity. Limits of detection (LOD) and limits of quantification (LOQ) were determined calculating analytes concentration for a signal/noise ratio (S/N) of 3 and 10 respectively.

Method precision was confirmed by repetitive analyses, calculating the average standard deviation (RSD) for six repeated determinations. For the recovery test, standard carotenoids solutions were added into egg yolk extracts in two concentrations levels and then analyzed by HPLC in triplicate. Recoveries for the seven carotenoids were then calculated as a percentage ratio between measured concentration and the added concentration.

8.3. Results and discussions

8.3.1. Linearity

Linearity of the method was evaluated as a function of response area. Calibration curves were obtained by injecting standard solutions in a concentration range of 2–20 mg/L.

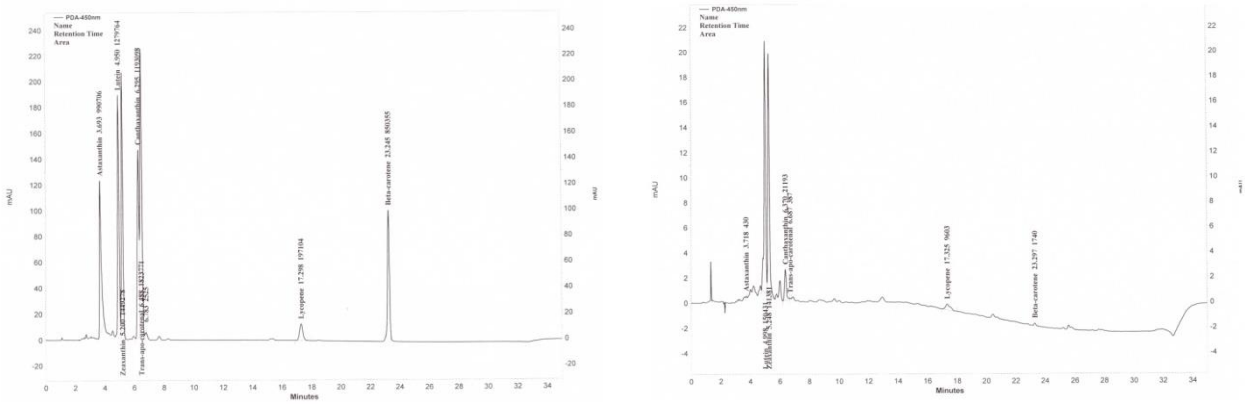

Figure 8.1. a. Chromatogram of a mix of standards at λ = 450 nm; b. Chromatogram at λ = 450 nm of an egg yolk extract.

Retention times, regression equations and correlation coefficients are presented in Table 8.1. Correlation coefficients of the linear regression analyze were above 0.997, and for the quantitative analyze was used the external standard method.

8.3.2. Reproducibility and detection limit

A solution made from a mix of standard with a concentration of 10 mg/L of every analyte was analyzed six times in order to determine the reproducibility of the peaks area and retention times in the optimal conditions for this experiment. Standard deviations for retention times ranged between 0.090 and 0.491%. These values indicated the stability of the method in terms of peaks area and retention times. Detection limits were between 0.041 and 0.752 mg/L for the seven carotenoid compounds (Table 8.1.).

8.3.3. Precision and stability

For testing the precision and stability of the HPLC method, a sample extract was analyzed by six repeated injections and the analyze was repeated after three days. For the retention times, the average values of the relative standard deviation (RSD) were between 0.081 and 0.755%, while for the peaks areas, RSD values ranged between 1.692 and 4.815%. The results proved that the HPLC method has a good precision and stability.

Table 8.1. Calibration curves for determination of the carotenoid compounds in egg yolk

Carotenoids	Retention time (min)	Regression equation		Concentrations interval (mg/L)	Coefficient of correlation r^2	Limit of detection (mg/L)
		Intersection	Slope			
Astaxanthin	3.71	-0.02353	8.19028 e-006	1.2-15	0.9997	0.398
Lutein	4.95	0	7.68226 e-006	0.9-15	0.9996	0.259
Zeaxanthin	5.20	0	6.81732 e-006	0.8-15	0.9994	0.236
Canthaxanthin	6.31	0	8.47711 e-006	2.5-20	0.9990	0.752
Trans-β-apo-8'-carotenal	6.51	0	5.34553 e-006	1.6-15	0.9997	0.490
Lycopene	17.29	0	4.46802 e-005	1.8-15	0.9970	0.522
β-Carotene	23.27	0	1.28077 e-005	0.2-20	0.9967	0.041

8.3.4. Sample analyze and recovery

Experiments for the recovery of the seven carotenoids were performed by adding carotenoid standards in the egg yolk extract at two levels of concentration, and analyzing six times according to the method described above. Recovery values ranged between 97.27 and 103.61%.

8.3. Partial conclusions

The developed reverse phase HPLC method represents an excellent technique for simultaneous determination of seven carotenoids in eggs yolk extracts, with a good sensitivity, precision and reproducibility. The method offers a good resolution of the analytes with a relatively short time of analyze (35 min). This method can be used for the quality control of the egg yolk and can play an essential role in understanding the influence of the diet and environment on the carotenoid content in the laying hens' yolk.

CHAPTER 9

STUDY REGARDING THE INCLUSION OF BY-PRODUCTS OF TOMATOES PROCESSING IN THE DIETS OF LAYING HENS

9.1. Study opportunity

Eggs are considered to be a valuable contributor to the overall nutritional balance of the diet, being recognised as a good source of high biological value proteins, essential fatty acids, vitamins and minerals [53]. Moreover, egg yolks serve as an important dietary source of highly bioavailable forms of the carotenoids lutein and zeaxanthin that are essential components for their antioxidant and immunomodulatory functions and for their positive health effects [54]. However, tomato by-products represent a rich souce of nutrients and biologically active compounds: carotenoids, proteins, polyphenols, minerals and oils. Although lycopene appears to have attracted only limited attention as a pigment for egg yolk, several studies investigated the effects of dietary lycopene or lycopene rich products supplementation on the performance and egg quality of laying hens [55, 56].

9.2. Materials and methods

9.2.1. Feeding Experiment

A 6-week feeding trial was conducted on 48 Tetra SL (53 weeks) layers, assigned to two groups: the control group received a basal diet (BD), while the experimental diet was supplemented with 7.5% dried tomato waste (DTW). The diets were balanced to be iso-nitrogenous and iso-caloric, and meet all other nutrient requirements of the hens.

9.2.2. Sampling and analyses

Feed consumption, egg production rate and egg weights were recorded daily. Initially, then every two weeks, 18 eggs per group were collected randomly. At each collection, the eggs were measured for the physical parameters: egg weight and the weight of the egg components (egg white, yolk, eggshell); colour intensity and Haugh units; eggshell thickness and eggshell breaking strength. For the determination of the carotenoids concentration in egg yolk, 10 eggs per group were collected randomly at the beginning of the trial, and every two weeks throughout the experimental period.

9.2.3. Extraction and HPLC analysis of carotenoids

Yolk samples were subjected to triplicate analyses for carotenoids using high-performance liquid chromatographic assay with diode-array detection at 450 nm, according to the method presented in chapter 8.2.

9.3. Results and discussion

The total carotenoid content in the basal diet was relatively low (2.08 mg/kg) compared with that of the diet supplemented with dried tomato waste (22.23 mg/kg). The dominating component of the carotenoid profile of the dried tomato waste supplemented diet (DTW) was lycopene (16.45 mg/kg) followed by β-carotene (3.15 mg/kg). In the control diet lutein was the major carotenoid with small levels of other carotenoids, including zeaxanthin (0.65 mg/kg) and β-carotene (0.27 mg/kg).

Supplementation with dried tomato waste significantly decreased the daily feed intake by 7.8. Although layers fed dried tomato waste had slightly lower egg production and feed conversion, the differences were found to be statistically non-significant in comparison with the control group. The average egg weight of hens that were fed the tomato waste supplemented diet was significantly higher than that of hens fed the basal diet. In addition, hens fed DTW showed slightly higher egg mass yield than control but the data did not differ significantly ($P>0.05$) from that of control.

The average egg weight of hens that were fed the tomato waste supplemented diet (6.6) was significantly higher than that of hens fed the basal diet (3.6). Egg yolk carotenoid concentrations significantly increased for hens given diets supplemented with dried tomato waste (Table 9.3.), suggesting the transfer of carotenoids including lycopene, the main carotenoid in dried tomato by-products, from feed to the egg.

Table 9.2. Effects of dried tomato waste supplementation to hens diets on performance and egg quality (over a 6 weeks period)*

Characteristics	Basal diet	Tomato waste diet
Feed intake (g/hen/day)	126.15 ± 0.836^{b}	118.32 ± 0.609^{a}
Hen-day egg production (%)	94.17 ± 0.841	92.29 ± 1.007
Feed conversion (g/g)	2.16 ± 0.020	2.04 ± 0.026
Egg weight (g)	64.04 ± 0.082^{b}	64.44 ± 0.111^{a}
Egg mass (g/hen/day)	55.44 ± 0.414	55.89 ± 0.474
Shell weight (g)	9.12 ± 0.096	8.97 ± 0.121
Shell thickness (mm)	0.34 ± 0.003^{b}	0.33 ± 0.003^{a}
Shell breaking strength (kg)	3.79 ± 0.100	3.76 ± 0.083
Haugh unit	77.17 ± 1.215	73.85 ± 1.507
Egg yolk weight (g)	16.65 ± 0.176	16.51 ± 0.147
Egg yolk colour	3.66 ± 0.216^{b}	6.60 ± 0.460^{a}

*means in the same row with different superscripts differ significantly ($P < 0.05$)

It is generally accepted that recovery of carotenoids in egg yolk from diet is conditioned not only by the ingested amount but also by the transfer rate. In addition, hens can metabolically transform certain carotenoids before deposition into yolk [57]. As a result, the pattern of carotenoid deposition into yolk reflects some interplay between a hen's diet and her capacity to absorb, transport, store and (or) modify carotenoids, while reconciling any competing somatic demands for their use (e.g. antioxidant protection, immune function) [58]. On the other hand, the deposition of carotenoids in yolks depends on their polarity, which is lower in nonpolar carotenes (lycopene and β-carotene) than in xanthophylls (lutein, zeaxanthin) that contain at least one atom of oxygen [59].

Table 9.3 Evolution of egg yolk carotenoid levels (µg/g) as affected by dried tomato waste supplementation of the hens diet* (BD = basal diet; DTW = dried tomato waste supplemented diet)

Characteristics	Initial	2 weeks	4 weeks	6 weeks
Astaxanthin				
BD	0.925±0.086	0.962±0.013^{a}	0.965±0.016	0.955±0.018^{a}
DTW		0.984±0.015^{b}	0.958±0.008	0.981±0.016^{b}
Lutein				
BD	2.967±0.239	2.432±0.449^{a}	3.453±0.478^{a}	2.135±0.197^{a}
DTW		4.311±0.055^{b}	4.226±0.415^{b}	4.469±0.079^{b}
Zeaxanthin				
BD	2.229±0.092	3.053±0.457^{a}	3.542±0.139^{a}	2.917±0.302^{a}
DTW		3.972±0.026^{b}	4.414±0.443^{b}	4.516±0.070^{b}
Canthaxanthin				
BD	0.084±0.019	0.077±0.009^{a}	0.125±0.015	0.198±0.019^{a}
DTW		0.092±0.010^{b}	0.143±0.017	0.338±0.011^{b}
Trans-β-apo-8'-carotenal				
BD	0.305±0.015	0.398±0.064^{a}	0.464±0.026	0.326±0.037^{a}
DTW		0.525±0.032^{b}	0.494±0.030	0.443±0.016^{b}
Lycopene				
BD	nd	nd^{a}	nd^{a}	nd^{a}
DTW		1.794±0.119^{b}	1.742±0.160^{b}	1.844±0.323^{b}
β-carotene				
BD	nd	Nd	nd^{a}	nd^{a}
DTW		Nd	0.065±0.020^{b}	0.071±0.010^{b}

*Means within each column for carotenoid compound and period bearing different superscripts differ significantly ($P < 0.05$); nd = not detected

The transfer rate TR (%) = {[yolk carotenoid concentration (µg/g) × yolk mass production (g/day/hen)] / [feed carotenoid concentration (µg/g) × feed intake (g/day/hen)]} × 100. In the present study a transfer rate of 5.3% was found for lycopene. Benakmoum et al. (2013) [60] reported transfer rates for lycopene between 1.86 and 3.48% after 3 to 13% dried tomato peel feeding, respectively. A higher transfer rate of 5.8% was reported by Karadas et al. (2006) [61] from a tomato powder supplemented diet.

9.4. Partial conclusions

Dried tomato waste supplementation to laying hens diet at 7.5% incorporation rate decreased the daily feed intake but had no detrimental effect on the rate of egg production. The egg weight of dried tomato waste supplemented hens increased compared with the control while egg mass output and egg quality was not significantly affected.

The total carotenoid concentration in the egg yolk increased by 1.5 times as a result of the use of dried tomato waste as forage material for laying hens while the egg yolk colour improved, reflecting the carotenoid content.

CHAPTER 10

STUDY ON THE SIMULTANEOUS INCLUSION IN LAYING HENS DIET OF BOTH FLAXSEEDS AND DRIED TOMATO BY-PRODUCTS

10.1. Study opportunity

Although the effects of the tomato by-products have been evaluated for laying hens, there are no studies on the effect of including them next to flaxseeds, which are rich in polyunsaturated fatty acids, in layer formulations. The purpose of this study was to evaluate the effects of the simultaneous inclusion of flaxseeds and dried tomato by-products (peels+seeds) in layer diets on hens' performance, egg quality characteristics, yolk carotenoids and polyunsaturated fatty acids content and yolk lipid peroxidation.

10.2. Materials and methods

The feeding trial was conducted in the experimental halls of The National Research-Development Institute of Animal Biology and Nutrition (IBNA-Balotesti, Romania) according to a protocol approved by the Commission of Ethics of the institute. The 6-week experiment used 96 Tetra SL layers (aged 53 weeks), assigned to four groups (C, E1, E2 and E3) (Table 10.1). The difference between the control diet (C) and the experimental diets (E1, E2, E3) came from the inclusion of 5% flax seeds and from the level of dry tomato waste in the diet formulations: 2.5% (E1); 5% (E2) and 7.5% (E3).

Table 10.1. Diet formulation and estimated chemical composition of the experimental diets

Diet composition [%]	C	E1	E2	E3
Corn	32.42	31.11	27.5	24.86
Wheat	25	25	25	25
Soy flour	22.2	22.8	22.3	21.85
Sunflower meal	6	-	-	-
Vegetable oil (soy beans)	2.81	1.8	3.35	3.92
Flax seeds	-	5	5	5
Dried tomatoes pomace	-	2.5	5	7.5
Lysine	-	0.04	0.07	0.06
Methionine	0.12	0.19	0.21	0.22
Calcium carbonate	8.85	8.87	8.87	8.88
Calcium phosphate	1.2	1.28	1.29	1.3
Sodium chloride (Salt)	0.35	0.36	0.36	0.36
Choline	0.05	0.05	0.05	0.05
Vitamins-minerals premix*	1	1	1	1
Chemical composition (calculated)				
Dry matter (DM) [%]	87.65	88.45	89.12	89.57
Metabolizable energy (ME) [kcal/kg]	2750	2750	2750	2750
Crude protein (CP) [%]	17.5	17.5	17.5	17.5
Crude fat (EE) [%]	4.23	4.77	6.27	6.81

Crude fiber (CF) [%]	4.32	5.48	6.9	8.11
Lysine [%]	0.86	0.84	0.84	0.84
Methionine [%]	0.41	0.44	0.45	0.46

*Supplied per kg diet: vitamin A, 13500 IU; vitamin D3, 3000 IU; vitamin E, 27 mg; vitamin K3, 2 mg; vitamin B1, 2 mg; vitamin B2, 4.8 mg; acid pantothenic, 14.85 mg; nicotinic acid, 27 mg; vitamin B6, 3 mg; vitamin B7, 0.04 mg; vitamin B9, 1 mg; vitamin B12, 0.018 mg; vitamin C, 25 mg; manganese (as manganese oxide), 71.9 mg; iron as (ferrous sulfate), 60 mg; cooper (as copper sulphate), 6 mg; zinc (as zinc oxide), 60 mg; cobalt (as cobalt sulphate), 0.5 mg; iodine (as potassium iodide), 1.14 mg; selenium (as sodium selenite), 0.18 mg.

Feed intake [g/layer/day], egg production and egg weight were monitored daily throughout the experiment. Laying percentage was calculated as number of eggs produced per hen divided by the number of days of the experimental period. Data on feed intake and egg mass were used to calculate feed conversion [feed intake/egg mass; g/g].

Yolk colour was determined every three days, on 5 eggs/group, by the Roche yolk colour fan. Every two weeks, 18 eggs/group were collected randomly from each group and used to determine the internal and external quality parameters of the eggs: weight of the egg and its components (albumen, yolk, shell), egg freshness and Haugh unit, eggshell thickness, and eggshell breaking strength.

To determine the fatty acid and carotenoid profile of egg yolk, every two weeks 10 eggs/group were collected randomly. Peroxide and TBARS values were used to monitor the oxidative stability of egg yolks during 28 days of storage at 4°C. After six weeks of experiment, 12 egg samples were collected for these measurements from C and E2 groups.

Yolk samples were subjected to triplicate analyses for carotenoids using high-performance liquid chromatographic assay with diode-array detection at 450 nm, as described by Corbu et al. (2017) [62]. Fatty acids content was assessed by fatty acid methyl ester (FAME) gas chromatography.

10.3. Results and discussions

10.3.1. Dietary ingredients and compound feeds

Fiber content was directly correlated with the level of dried tomato waste inclusion in the compound feeds. In all three experimental formulations (E1, E2, E3), lycopene was the dominant component of the carotenoid profile of the compound feed, followed by β-carotene and lutein (table 10.3).

The increase of carotenoid concentration in the experimental feeds was positively correlated with the amount of dietary dried tomato waste. The total carotenoid content of the experimental formulations was 4.61 (E1), 9.21 (E2) and 11.88 (E3) times higher than in C.

The addition of flaxseeds to the supplemented feeds made the n-3 PUFA concentration to increase about 9 times compared to the control feed, also improving the n-6/n-3 ratio (Table 10.3).

Table 10.3. Average chemical composition of the experimental compound feeds

Specification	C	E1	E2	E3
Basic chemical composition of the compound feeds [%]				
Dry matter (DM) [%]	89.19	89.49	90.01	90.40
Crude protein (CP) [%]	18.08	17.30	17.40	17.20
Crude fat (EE) [%]	4.38	4.75	6.33	7.04
Crude fibre (CF) [%]	5.79	6.46	7.04	7.98
Ash [%]	13.34	13.07	12.92	12.12
Polyunsaturated fatty acids (PUFA) profile				
Total PUFA [g/100g total fatty acids], of which:	55.28	57.76	58.57	57.62
n-6 PUFA	53.88	44.59	45.54	44.64
n-3 PUFA	1.40	13.19	13.03	12.98
n-6/n-3	38.39	3.38	3.42	3.44
Carotenoid profile				
Astaxanthin [mg/kg]	-	0.03	0.02	0.03
Lutein [mg/kg]	0.80	1.04	1.44	1.47
Zeaxanthin [mg/kg]	0.65	0.78	0.88	0.84
Canthaxanthin [mg/kg]	0.09	0.11	0.15	0.16
Trans-apo-carotenal [mg/kg]	0.10	0.10	0.11	0.11
Lycopene [mg/kg]	0.24	6.62	14.62	19.69
Beta-carotene [mg/kg]	0.27	1.27	2.64	3.33
Total carotenoid content [mg/kg]	2.16	9.95	19.87	25.62

SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids.

Table 10.4. Oxidative stability of the compound feeds at 0, 14 and 28 days of storage

	Days of storage	C	E1	E2	E3	Maximum limits*
Peroxide value [ml thyosulphate 0.1 N/g fat]	0	5.1	5.4	5.0	5.1	12
	14	6.0	6.7	6.0	6.0	
	28	8.3	8.3	8.6	8.7	
Fat acidity [mg KOH/g fat]	0	15.15	16.61	14.40	13.11	50
	14	17.21	18.19	17.14	17.22	
	28	19.36	19.52	20.56	20.55	
Kreis test	0	negative	negative	negative	negative	negative
	14	negative	negative	negative	negative	
	28	negative	negative	negative	negative	

* According Romanian regulation (Order no. 249/358 of the Ministry of Agriculture, Food and Forestry published on 31 March 2003)

10.3.2. Layer performance and egg quality

The layers from the groups supplemented with 5% and 7.5% dried tomato waste (E2 and E3) had a significantly ($p < 0.05$) lower average daily feed intake than C and E1. The layers

from the control group had a lower feed conversion ratio than those from the experimental groups, although the differences were not statistically significant ($p > 0.05$).

The lower average daily feed intake influenced the laying percentage, which was significantly ($p < 0.05$) lower in groups E2 (87.92%) and E3 (90.21%) compared to C. The average weight of the eggs and of their components (albumen, yolk and shell), as well as the shell breaking strength, were not significantly different between groups ($p < 0.05$) (Table 10.5).

Table 10.5. Influence of the dietary flaxseeds and dried tomato waste on layer performance and egg quality *

Specification	C	E1	E2	E3	SEM	p-value
Initial weight [g/layer]	1967.5	1966.3	1967.1	1967.1	15.4	>0.999
Final weight [g/layer]	2029.2	2055.8	2083.3	2067.5	19.0	0.786
Average daily feed intake [g/layer/day]	126.1^{a}	127.3^{a}	120.2^{b}	123.6^{c}	0.4	<0.0001
Feed conversion ratio	2.2^{a}	2.2^{b}	2.2^{ab}	2.2^{ab}	0.01	0.065
[g/g egg]	94.2^{a}	92.7^{ac}	87.9^{bc}	90.2^{c}	0.49	<0.0001
Laying percentage [%]	64.6	64.4	64.4	64.5	0.2	0.928
Average egg weight [g],	38.8	38.7	38.6	39.2	0.17	0.656
of which:	16.6	16.5	16.7	16.5	0.07	0.749
- albumen	9.1	9.1	9.0	9.0	0.05	0.629
- yolk	7.7	7.8	7.8	7.8	0.01	0.459
- shell	5.8	5.8	5.8	5.8	0.02	0.9685
Albumen pH	0.34	0.34	0.34	0.34	0.001	0.04
Yolk pH	3.8	3.5	3.6	3.7	0.04	1.81
Eggshell thickness [mm]	77.2	75.9	73.9	76.5	0.54	0.176

*Mean values within a row having different superscripts are significantly different by least significant difference test ($p < 0.05$); SEM: standard error of the mean.

10.3.3. Egg colour

Starting with experimental day 3, the yolk colour score was significantly ($p < 0.05$) higher in the eggs from the groups treated with flaxseeds and dried tomato waste, the strongest yolk pigmentation being noticed in the yolk of E3 eggs (7.5% dried tomato waste).

10.3.4. Egg yolk PUFA and carotenoid content

n-3 PUFA concentration was significantly ($p < 0.05$) higher in the yolk from the experimental groups compared to group C: 3.69-fold for E1, 3.34-fold for E2, and 3.15-fold for E3. In addition, the n-6/n-3 ratio decreased from 18.33 (C) to 4.13 (E1), 4.99 (E2) and 5.43 (E3).

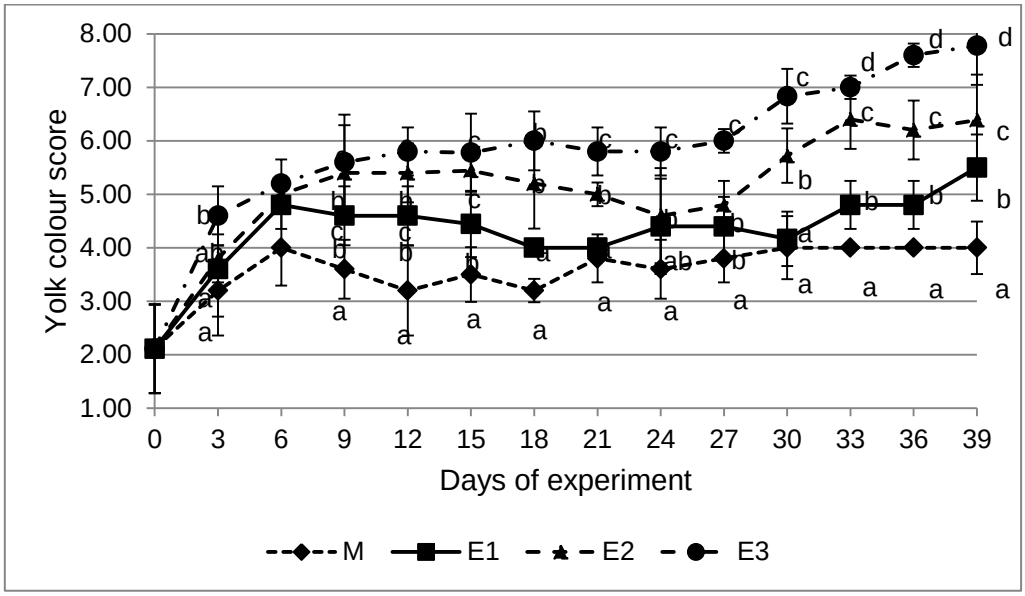

Figure 10.1. Evolution of egg yolk colour, measured by the Roche colour fan. Different letters within the same day indicate significant differences ($P < 0.05$) among experiment.

Egg yolk carotenoid concentrations significantly increased for hens given diets supplemented with dried tomato waste (Table 10.7), suggesting the transfer of carotenoids from feed to egg.

Yolk colour correlated positively and significantly ($r = 0.71$, $p < 0.05$) with carotenoids content. However, the transfer efficacy of carotenoids to egg yolk and their influence on yolk coloration differs greatly depending upon the type of carotenoids present and the chemical form of the molecules [63]. Chicken is characterized by the almost exclusive accumulation of xanthophylls that are the major contributor to the pigmentation of chicken egg yolk as β-carotene is almost completely converted into vitamin A or is otherwise metabolized [64].

The increase in dried tomato waste content of the diet has depressed the absorption and deposition of n-3 fatty acids in egg yolk. Similar findings were reported by Galobart et al. (2001a) [65] and Meluzzi et al. (2000) [66] who observed a reduction of the n-3 fatty acids content in eggs enriched with ω3-polyunsaturated fatty acids from α-tocopherol supplemented treatments. They suggested that α-tocopherol at high doses can interfere in the intestinal absorption of some long-chain fatty acids or that it can act as a pro-oxidant in eggs. It is well known that, at high concentrations, carotenoids may act as pro-oxidants in biological systems [67] but more research is needed to elucidate the effect of these bioactive compounds on the fatty acids composition of eggs enriched with ω3-polyunsaturated fatty acids.

After 4 experimental weeks, lutein and zeaxanthin concentrations in egg yolk from E3 group increased by 62% and 39% respectively, compared to C group. Lycopene, the main carotenoid in tomato waste, was determined only in the yolk of the eggs from the experimental groups, but its concentration reached a maximum of only 1.76 mg/kg after 4 weeks of feeding with the diet supplemented with 7.5% dried tomato waste. It is generally accepted that recovery of carotenoids in egg yolk from diet is conditioned not only by the ingested amount but also by the transfer rate. In addition, hens can metabolically transform certain carotenoids before deposition into yolk [57].

Table 10.6. Total PUFA content [g/100g total fatty acids] of egg yolk from laying hens fed different levels of flaxseed and dried tomato waste *

	Weeks of feeding	C	E1	E2	E3	SEM	p-Value
Total PUFA, of which:	0	30.58					
	2	29.02^{a}	28.91^{a}	31.05^{b}	32.16^{c}	0.298	<0.0001
	4	28.20^{a}	27.71^{a}	30.76^{b}	32.40^{c}	0.411	<0.0001
	6	28.65^{a}	28.73^{a}	30.73^{b}	31.88^{c}	0.302	<0.0001
n-3 PUFA	0	2.27					
	2	1.63^{a}	5.65^{b}	5.05^{c}	4.68^{d}	0.327	<0.0001
	4	1.47^{a}	5.69^{b}	5.16^{c}	4.80^{d}	0.349	<0.0001
	6	1.49^{a}	5.61^{b}	5.13^{c}	4.97^{c}	0.359	<0.0001
n-6 PUFA	0	28.31					
	2	27.39^{a}	23.26^{b}	26.00^{c}	27.48^{a}	0.361	<0.0001
	4	26.24^{a}	22.50^{b}	25.60^{c}	27.60^{d}	0.396	<0.0001
	6	27.16^{a}	23.1^{b}	25.59^{c}	26.99^{a}	0.355	<0.0001
n-6/n-3	0	12.71					
	2	16.81^{a}	4.14^{b}	5.15^{c}	5.88^{d}	1.074	<0.0001
	4	17.95^{a}	3.97^{b}	4.97^{c}	5.77^{d}	1.190	<0.0001
	6	18.33^{a}	4.13^{b}	4.99^{c}	5.43^{c}	1.272	<0.0001

* Mean values within a row having different superscripts are significantly different by least significant difference test ($p < 0.05$). SEM: standard error of the mean; PUFA: polyunsaturated fatty acids.

Table 10.7. Carotenoid concentration in the fresh yolk samples [mg/kg, average values/group]*

	Weeks of feeding	C	E1	E2	E3	SEM	p-Value
Astaxanthin	0	0.925					
	2	0.96	0.98	0.97	0.97	0.004	0.4308
	4	0.96	0.96	0.96	0.96	0.003	0.8655
	6	0.95	0.95	0.96	0.96	0.003	0.4268
Lutein	0	2.967					
	2	2.43^{a}	3.11^{b}	4.14^{c}	4.18^{c}	0.186	<0.0001
	4	2.45^{a}	3.58^{b}	3.69^{b}	3.98^{c}	0.148	<0.0001
	6	2.13^{a}	2.50^{b}	3.22^{c}	3.42^{c}	0.130	<0.0001
Zeaxanthin	0	2.229					
	2	3.05^{a}	2.71^{a}	4.57^{c}	4.04^{b}	0.184	<0.0001
	4	3.54^{a}	3.75^{a}	3.91^{ab}	4.93^{b}	0.141	<0.0001
	6	2.91^{a}	3.13^{ab}	3.15^{ab}	3.39^{b}	0.066	0.0817
Cantaxanthin	0	0.084					
	2	0.08^{a}	0.11^{bc}	0.09^{b}	0.11^{c}	0.004	0.0010
	4	0.12^{a}	0.15^{b}	0.15^{b}	0.14^{ab}	0.004	0.0397
	6	0.20^{a}	0.22^{ab}	0.27^{b}	0.37^{c}	0.017	<0.0001
Trans-apo-carotenal	0	0.305					
	2	0.40^{a}	0.42^{a}	0.51^{b}	0.56^{b}	0.017	<0.0001

	4	0.46^{a}	0.52^{ab}	0.51^{ab}	0.58^{b}	0.014	0.0141
	6	0.33^{a}	0.32^{a}	0.34^{a}	0.44^{b}	0.015	0.0034
Lycopene	0	Nd					
	2	nd^{a}	nd^{a}	0.90^{b}	1.19^{b}	-	-
	4	nd^{a}	0.63^{b}	1.33^{c}	1.76^{d}	0.136	<0.0001
	6	nd^{a}	0.30^{b}	0.92^{c}	1.63^{d}	0.105	<0.0001
Beta-carotene	0	Nd					
	2	nd^{a}	nd^{a}	nd^{a}	0.05^{b}	-	-
	4	nd^{a}	nd^{a}	0.06^{b}	0.06^{b}	0.136	<0.0001
	6	0.04^{a}	0.04^{a}	0.04^{a}	0.06^{b}	0.002	0.0002

* Mean values within a row having different superscripts are significantly different by least significant difference test ($p < 0.05$). SEM: standard error of the mean; ud = undetected.

10.3.5. Egg yolk lipid peroxidation

After both 14 and 28 days of storage, peroxide values and TBARS values were significantly lower in egg yolk from hens fed the diet with 5% flaxseeds and 5% dried tomato waste than in those from the control, despite the fact that n-3 fatty acids-enriched eggs are more susceptible to lipid oxidation, as it is well documented in literature [68].

10.3.6. Lipid peroxidation of compound feeds

The high concentration of unsaturated fatty acids in feeds favors lipid peroxidation. Numerous earlier studies reported that animal performance is affected by feeding peroxidized lipids [69]. Although peroxide value and fat acidity were slightly higher in the diets supplemented with flaxseeds compared to the control diet, their values were below the maximum limits set for compound feeds according the Romanian Standard STAS 12266-84, after both storage periods (14 and 28 days). Kreis test was negative in all samples throughout the period of study. These results are probably due to the antioxidant carotenoids coming from dried tomato waste.

10.4. Partial conclusions

The simultaneous supplementation of the hens diet with flaxseeds and dried tomato waste led to obtaining the so-called “designer eggs” that are high in essential n-3 fatty acids and carotenoids. The use of flaxseeds in layer diets increased significantly ($p < 0.05$) the n-3 PUFA concentration in the egg yolk.

Flaxseeds and dried tomato waste supplementation to laying hens diet at 5% and 7.5% respectively decreased the daily feed intake but had no detrimental effect on the average weight of the eggs and of their components (albumen, yolk and shell). Compound feeds supplemented with flaxseeds are highly susceptible to oxidation but addition of tomato waste limited their oxidative deterioration.

Adding tomato waste to the layers’ diet resulted in a significant increase of the Roche colour score in correlation with the increased deposition of carotenoids in egg yolks. Dietary supplementation with 5% dried tomato waste increased the oxidative stability of n-3 PUFA enriched eggs but the increase in the dried tomato waste content of the diet has depressed the absorption and deposition of n-3 fatty acids in egg yolk.

CHAPTER 11

FINAL CONCLUSIONS

→ By-products resulted after tomatoes processing (peels + seeds) showed a high nutrition value determined by the content of essential amino acids, polyunsaturated fatty acids, carotenoid compounds and mineral substances. These by-products contained in average 176.2 g/kg proteins, 21.9 g/kg fat, 524.4 g/kg brute fiber and 42.1 g/kd ash. Essential amino acids represented 34.2% of total protein, most abundant being leucine, followed by lysine and isoleucine. Unsaturated fatty acids represented 77.04% of the total fatty acids, most predominant being linoleic acid. Results confirmed that dehydrated tomato by-products contain important quantities of lycopene (510.6 mg/kg) and β-carotene (95.6 mg/kg) and present a strong antioxidant activity. Total phenolic content was 1229.5 mg GAE/kg, from which flavonoids represented 415.3 mg QE/kg. Ellagic and clorogenic acids were the most abundant phenolic acids, while among flavonoids only rutin and myricentin have been quantified;

→ By-products resulted after sea buckthorn processing for juice production represents a secondary product extremely valuable which, besides other important nutrients, contain also high quality lipids. These demonstrated a high nutritional value given by the high content of valuable fats (20.05%) and valuable proteins (14.89%). These by-products had also a content of fibers of 198.6 g/kg and 18.4 g/kg of ash. Essential amino acids represented 38.42% from total content of amino acids, in highest quantity being leucine (11.6 g/kg) followed by phenylalanine and lysine. Fatty acids profile showed a high concentration of monounsaturated fatty acids (5.08 of the total fatty acids), due to the high content of oleic and palmitoleic acids, a content of poly unsaturated fatty acids of 15.70% and a low n-6/n-3 ratio of fatty acids (1.42). Total average content of carotenoids was 245.6 mg/100 g, from which the most important were β-carotene (80.76 mg/kg) and zeaxanthin (69.60 mg/kg). Moreover, by-products from sea buckthorn fruits had a high content of phenolic compounds and a high antioxidant activity. Results shown that dehydrated sea buckthorn by-products are valuable sources of nutrients and bioactive compounds and have the potential for being used as nutraceutic for feed, as ingredients for functional foods, as well as in pharmaceutical industry.

→ Direct extraction of carotenoids from dehydrated tomato by-products in vegetable oil led to obtaining functional colored oil, with a high content of carotenoids and a high antioxidant activity. As the results have shown, carotenoids content in oils increased significantly by increasing the extracted quantity of dehydrated tomato by-products. Maceration proved as well able to extract significantly higher quantities of these compounds. In some oils, extraction from dehydrated tomato by-products improved oils oxidative and thermal stability, while in other oils led to an increase of peroxide index and a decrease of induction time. In some oils, results have shown pro-oxidative effects of the compounds extracted from dehydrated tomatoes by-products;

→ Extraction of sea buckthorn by-products in vegetable oils increased significantly the carotenoids content in oil and contributed to the increase of their antioxidant activity. Ultrasound assisted extraction was more efficient than maceration for direct extraction in oils of carotenoids compounds from secondary sea buckthorn products. Thermal stability of sunflower oil and olive oil wasn't very much affected by the enrichment with carotenoids extracted from sea buckthorn products, while the termokinetic stability was reduced. By-products resulted from sea buckthorn processing, after dehydration, can be used for direct extraction of carotenoids in edible oils for increasing oil functionality but also for their color effect and increase of the acceptability of the oils;

→ The reverse phase HPLC method developed and validated allowed simultaneous determination of seven carotenoid compounds (lutein, zeaxanthin, canthaxanthin, astaxanthin, lycopene, β-carotene and trans-β-apo-8'-carotenal) in egg yolk extracts, with a good sensitivity, precision and reproducibility. The method offers a good resolution of the analytes with a relatively short time of analysis (35 min). In the performed experiments, the developed method allowed to study the effects of supplementation of the laying hens diet with by-products resulted after tomatoes processing on the carotenoid content in egg yolk;

→ Supplementation of the diet of laying hens with dried tomato by-products at a level of incorporation of 7.5% reduced the daily feed intake but did not affected significantly the production of eggs and eggs quality. Weight of the hens that had a diet supplemented with dehydrated tomato by-products increased in comparison with those from the control group. After six weeks, carotenoids content in egg yolk increased 1.5 folds as a result of supplementation of hens diet with dehydrated tomato by-products, in direct correlation with the improvement of egg yolk color illustrated by the increase of Roche scale value from 3.66 to 6.60;

→ Simultaneous supplementation of laying hens diet with both flaxseeds and dehydrated tomato by-products led to obtaining of the so called “designer eggs”, enriched in n-3 polyunsaturated fatty acids and in carotenoids. Supplementation with 5% flaxseeds and 7.5% dehydrated tomato by-products determined the decrease of daily feed consumption but did not affect average values of egg weight and its components (albumen, yolk and shell). Compound feeds supplemented with flaxseeds are highly susceptible to oxidation but addition of tomato waste limited their oxidative deterioration;

→ Increase of the quantity of dehydrated tomatoes by-products in diet led to the increase of Roche color value scale in direct correlation with the enrichment of carotenoids content in egg yolk but decreased the efficiency of their transfer from feed to egg. After 4 weeks, egg yolk from hens fed with the supplemented feed with 5% flaxseeds and 7.5% dehydrated tomato by-products had increased levels of lutein and zeaxanthin (with 29% and 24% respectively) and the color score value was 3.5 folds higher when compared to those of the control group. As a result of supplementation of hens diet with flaxseeds, the content of n-3 fatty acids was 3.15-3.69 folds higher when compared to control group and the ratio n-6/n-3 decreased from 18.33 (control) to 4.13 (2.5 DTW), 4.99 (5% DTW) and 5.43 (7.5% DTW) respectively. Supplementation of hens’ diet with dehydrated tomato by-products reduced the lipid oxidation in eggs enriched with n-3 PUFA by diet supplementation with flaxseeds but the increase of the content of dehydrated tomato by-products in diet reduced the absorption and deposition of n-3 fatty acids in egg yolk.

CHAPTER 12

CONTRIBUTIONS AND PERSPECTIVES FOR FURTHER RESEARCH

According to the original experimental results obtained in this PhD thesis, the following can be highlighted as scientific contributions:

→ Determination of the content of nutritional substances and biologically active compounds from by-products resulted during tomatoes processing in order to develop new alternatives for the recovery of this valuable secondary product;

→ Assessment of dried sea buckthorn pomace for determining nutritional, bioactive and antioxidant properties in order to promote the consumption and usage in the food industry of this extremely valuable secondary products;

→ Usage of different vegetable oils as alternative extraction solvents for carotenoid compounds from by-products of the tomatoes processing in order to obtain oils enriched with carotenoids to be used in different food applications. Comparative studies have been performed for assessing the efficacy of carotenoids extraction from tomato by-products, and also upon color, thermal and oxidative stability of the oils obtained after extractions;

→ Usage of the secondary products resulted from sea buckthorn fruits processing as a natural source of carotenoids for enrichment of vegetable oils. Maceration and ultrasounds assisted extraction have been used for carotenoids extraction directly in oils. The resulting oils were characterized for total carotenoid content, antioxidant activity, color, thermal and oxidative stability;

→ Development and validation of a HPLC method for simultaneous determination of carotenoid compounds in egg yolk;

→ Obtaining of carotenoids enriched eggs by supplementation of the hens' diet with dehydrated tomato processing by-products;

→ Assessment of the effects generated by the simultaneous inclusion in laying hens diet of both flaxseeds and dehydrated tomato by-products (peels + seeds), on carotenoids and PUFA content in egg yolk as well as the degree of oxidation of the feed and egg yolk lipids;

→ Elaboration and patenting of a feed recipe for laying hens, in order to obtain, in a natural way, eggs with improved nutritional value by increasing the concentrations of n-3 PUFA and carotenoids of egg yolk by comparison with conventional produced eggs;

→ Realization and patenting of hens eggs with synergetic composition ("designer eggs") with a high content in yolk of n-3 fatty acids and antioxidant carotenoids that can be produced, in a stable way, by feeding laying hens with a traditional feed for laying hens supplemented with flaxseeds reach in n-3 fatty acids and with dehydrated tomato processing by-products (peels + seeds) that contain carotenoids in high concentrations. The high carotenoid content assures an antioxidant effect, reducing the n-3 fatty acids oxidation and offering to the human body an increased antioxidant intake.

The results obtained can represent the starting point for further development of the researches regarding recovery of by-products and wastes resulted during tomatoes and sea buckthorn fruits processing in products with an added value and the improvement of wastes management.

CHAPTER 13

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

Articles / studies published in ISI listed journals

1. Nour V., Corbu A.R., Rotaru P., Karageorgou I., Lalas S. 2018. Effect of carotenoids, extracted from dry tomato waste, on the stability and characteristics of various vegetable oils. Grasas y Aceites, 69 (1), e238, ISSN-L: 0017-3495, https://doi.org/10.3989/gya.0994171 (IF=0.891)

2. Nour V., Panaite D.T., Ropota M., Turcu R., Trandafir I., Corbu R.A. 2018. Nutritional and bioactive compounds in dried tomato processing waste, CyTA - Journal of Food, 16(1), 222-229 https://doi.org/10.1080/19476337.2017.1383514 http://www.tandfonline.com/doi/full/10.1080/19476337.2017.1383514 (IF=1,371)

3. Corbu A.R., Rotaru A., Nour V. 2019. Edible vegetable oils enriched with carotenoids extracted from by-products of sea buckthorn (*Hippophae rhamnoides* ssp. *sinensis*): the investigation of some characteristic properties, oxidative stability and the effect on thermal behavior. Journal of Thermal Analysis and Calorimetry. DOI:10.1007/s10973-019-08875-5 (IF=2.471)

4. Panaite T.D., Nour V., Vlaicu P.A., Ropota M., Corbu A.R., Saracila M. 2019. Flaxseed and dried tomato waste used together in laying hens diet. Archives of Animal Nutrition, 73(3), 222-238, https://doi.org/10.1080/1745039X.2019.1586500 https://www.tandfonline.com/doi/pdf/10.1080/1745039X.2019.1586500 (IF=1,887)

Articles / studies published in Web of Science / ISI Proceedings

1. Nour V., Panaite T.D., Vlaicu P.A., Corbu A.R. 2018. Responses of laying hens to the simultaneous dietary supplementation with flaxseed and dried tomato by-products. Journal of Biotechnology 280, S57. https://www.sciencedirect.com/science/article/pii/S0168165618303626

2. Panaite T.D., Criste R., Nour V., Saracila M., Vlaicu P.A., Ropota M., Corbu A.R. 2018. Effect of carotenoids on egg yolk fat lipid peroxidation. Journal of Biotechnology 280, S54. https://www.sciencedirect.com/science/article/pii/S0168165618303493

3. Nour V., Panaite T.D., Corbu A.R., Vlaicu P.A. 2017. Yolk colour and carotenoid composition of the eggs produced by laying hens fed diets containing tomato processing waste. SGEM International Multidisciplinary Scientific GeoConference, 27-30 November, 2017, Viena, Austria. Conference Proceedings, ISSN 1314-2704. https://www.sgem.org/index.php/elibrary?view=publication&task=show&id=4684

4. Nour V., Tuțulescu F., Ionica M.E., Corbu A. R. 2017. Dough reology and properties of gluten-free rice breads as affected by addition of hydrocolloids and emulsifiers. Carpathian Journal of Food Science and Technology, 9(4), 158-166. http://chimie-biologie.ubm.ro/carpathian_journal/Vol_9(4)_2017.pdf

5. Panaite T., Ropota M., Turcu R., Olteanu M., Corbu A.R., Nour V. 2017. Flaxseeds: Nutritional Potential and Bioactive Compounds. Bulletin UASVM Food Science and Technology 74(2), 65-73.
http://journals.usamvcluj.ro/index.php/fst/article/view/12762/pdf

Articles / studies published in journals indexed in international BDI databases

1. Corbu A.R., Nour V. 2017. Development and evaluation of a reverse-phase HPLC method for the analysis of carotenoids in egg yolk. Analele Universităţii din Craiova, seria Biologie, Horticultură, Tehnologia Prelucrării Produselor Agricole, Ingineria Mediului, vol. XXII (LVIII), 81-88.
http://horticultura.ucv.ro/horticultura/sites/default/files/horticultura/Reviste/Analele/2017/anale2017_sectiuneai_pp_1_340.pdf

Articles communicated at international scientific sessions

1. Nour V., Panaite T.D., Vlaicu P.A., Corbu A.R. 2018. Responses of laying hens to the simultaneous dietary supplementation with flaxseed and dried tomato by-products. European Biotechnology Congress, Athens, Greece 24-28th of April 2018.
2. Panaite T.D., Criste R., Nour V., Saracila M., Vlaicu P.A., Ropota M., Corbu A.R. 2018. Effect of carotenoids on egg yolk fat lipid peroxidation. European Biotechnology Congress, Athens, Greece 24-28th of April 2018.
3. Nour V., Panaite T.D., Corbu A.R., Vlaicu P.A. 2017. Yolk colour and carotenoid composition of the eggs produced by laying hens fed diets containing tomato processing waste. SGEM International Multidisciplinary Scientific GeoConference, 27-30 November, 2017, Viena, Austria.
https://www.sgem.org/sgemlib/spip.php?article11262

Articles communicated at national scientific sessions

1. Corbu A.R., Nour V. 2018. Enrichment of edible vegetable oils with carotenoids by extraction from sea buckthorn (*Hippophae rhamnoides* ssp. *sinensis*) by-products. Scientific Conference of Doctoral Schools. SCDS-UDJG 2018, The Sixth Edition, Galaţi, 7th-8th of June 2018.
http://www.cssd-udjg.ugal.ro/index.php/2018
2. Corbu A.R. Lazăr N., Nour V. 2018. Efectul carotenoizilor extrași din subproduse de la procesarea cătinei asupra stabilității oxidative și culorii unor uleiuri vegetale. Conferința Națională Studențească Provocări și oportunități privind valorificarea deșeurilor agro-alimentare, 17-18 mai 2018, Universitatea “Lucian Blaga” din Sibiu
http://saiapm.ulbsibiu.ro/wp-content/uploads/2018/05/Programul_CNSD_2018.pdf
3. Corbu A.R., Nour V. 2019. Nutritional and bioactive compounds in dried sea-buckthorn pomace. Scientific Conference of Doctoral Schools. SCDS-UDJG 2020, The 7th Edition, Galaţi, 13th-14th of June 2019.
http://www.cssd-udjg.ugal.ro/index.php/2019/programme-2019
4. Corbu A.R., Panaite T.D., Sărăcilă M., Nour V. 2020. Yolk colour and carotenoid composition of the eggs produced by laying hens fed diets supplemented with dried sea-buckthorn pomace. Scientific Conference of Doctoral Schools. SCDS-UDJG 2020, The 8th Edition, Galaţi, 18th-19th of June 2020.
http://www.cssd-udjg.ugal.ro/index.php/programme-2020

BIBLIOGRAPHY

[1]. Kosseva, M. R. (2011). Management and processing of food wastes. In: *Reference Module in Earth Systems and Environmental Sciences Comprehensive Biotechnology*, second ed., pp. 557–593.

[2]. Larrauri, J. A. (1999). New approaches in the preparation of high dietary fibre powders from fruit by-products. *Trends in Food Science & Technology*, *10*(1), 3-8.

[3]. Technologico, G. C. (2004). Handbook for the Prevention and Minimization of Waste and Valorisation of By-products in European Agro-food Industries.

[4]. Ayala-Zavala, J., Vega-Vega, V., Rosas-Domínguez, C., Palafox-Carlos, H., Villa-Rodriguez, J. A., Siddiqui, M. W., ... & González-Aguilar, G. A. (2011). Agro-industrial potential of exotic fruit byproducts as a source of food additives. *Food Research International*, *44*(7), 1866-1874.

[5]. Grigoras, C. G., Destandau, E., Lazar, G., & Elfakir, C. (2012). Bioactive compounds extraction from pomace of four apple varieties. *Journal of Engineering Studies and Research*, 18(1), 96-103.

[6]. Górnaś, P., Rudzińska, M., Raczyk, M., & Soliven, A. (2016). Lipophilic bioactive compounds in the oils recovered from cereal by-products. *Journal of the Science of Food and Agriculture*, *96*(9), 3256-3265.

[7]. Shalini, R., & Gupta, D. K. (2010). Utilization of pomace from apple processing industries: a review. *Journal of Food Science and Technology*, *47*(4), 365-371.

[8]. FAOSTAT database (2015). Food and Agriculture Organization of the United Nations. Available at http://faostat3.fao.org/browse/Q/QC/E.

[9]. Knoblich, M., Anderson, B., & Latshaw, D. (2005). Analyses of tomato peel and seed byproducts and their use as a source of carotenoids. *Journal of the Science of Food and Agriculture*, *85*(7), 1166-1170.

[10]. Al-Weshahy, A., El-Nokety, M., Bakhete, M., & Rao, V. (2013). Effect of storage on antioxidant activity of freeze-dried potato peels. *Food Research International*, *50*(2), 507-512

[11]. Jun, X. (2006). Application of high hydrostatic pressure processing of food to extracting lycopene from tomato paste waste. *High Pressure Research*, *26*(1), 33-41.

[12]. Choudhari, S. M., & Ananthanarayan, L. (2007). Enzyme aided extraction of lycopene from tomato tissues. *Food Chemistry*, *102*(1), 77-81.

[13]. Lavecchia, R., & Zuorro, A. (2008). Improved lycopene extraction from tomato peels using cell-wall degrading enzymes. *European Food Research and Technology*, *228*(1), 153.

[14]. Papaioannou, E. H., & Karabelas, A. J. (2012). Lycopene recovery from tomato peel under mild conditions assisted by enzymatic pre-treatment and non-ionic surfactants. *Acta Biochimica Polonica*, *59*(1), 71-74.

[15]. Baysal, T., Ersus, S., & Starmans, D. A. J. (2000). Supercritical CO2 extraction of β-carotene and lycopene from tomato paste waste. *Journal of Agricultural and Food Chemistry*, *48*(11), 5507-5511.

[16]. Topal, U., Sasaki, M., Goto, M., & Hayakawa, K. (2006). Extraction of lycopene from tomato skin with supercritical carbon dioxide: effect of operating conditions and solubility analysis. *Journal of Agricultural and Food Chemistry*, *54*(15), 5604-5610.

[17]. Salta, F. N., Mylona, A., Chiou, A., Boskou, G., & Andrikopoulos, N. K. (2007). Oxidative stability of edible vegetable oils enriched in polyphenols with olive leaf extract. *Food Science and Technology International*, *13*(6), 413-421.

[18]. Tsaknis, J., & Lalas, S. (2005). Extraction and identification of natural antioxidant from *Sideritis euboea* (mountain tea). *Journal of Agricultural and Food Chemistry*, *53*(16), 6375-6381.

[19]. Moure, A., Cruz, J. M., Franco, D., Domínguez, J. M., Sineiro, J., Domínguez, H., & Parajó, J. C. (2001). Natural antioxidants from residual sources. *Food Chemistry*, *72*(2), 145-171.

[20]. Beveridge, T., Li, T. S., Oomah, B. D., & Smith, A. (1999). Sea buckthorn products: manufacture and composition. *Journal of Agricultural and Food Chemistry*, *47*(9), 3480-3488.

[21]. Mironov, V. A. (1989, October). Chemical composition of Hippophae rhamnoides of different populations of the USSR. In *Proceedings of International Symposium on sea buckthorn* (pp. 80-87).

[22]. Yang, B., & Kallio, H. (2002). Composition and physiological effects of sea buckthorn (*Hippophae*) lipids. *Trends in Food Science & Technology*, *13*(5), 160-167.

[23]. Schroeder, W. R., & Yao, Y. (1995). *Sea buckthorn: A promising multipurpose crop for Saskatchewan*. PFRA Shelterbelt Centre.

[24]. Dharmananda, S. (2004). Sea buckthorn, Institute of Traditional Medicine, Portland, Oregon.

[25]. Rösch, D., Krumbein, A., & Kroh, L. W. (2004). Antioxidant gallocatechins, dimeric and trimeric proanthocyanidins from sea buckthorn (*Hippophae rhamnoides*) pomace. *European Food Research and Technology*, *219*(6), 605-613.

[26]. Khiari, Z., Makris, D. P., & Kefalas, P. (2009). An investigation on the recovery of antioxidant phenolics from onion solid wastes employing water/ethanol-based solvent systems. *Food and Bioprocess Technology*, *2*(4), 337.

[27]. Enser, M. (2001). The role of fats in human nutrition. In B. Rossell (Ed.), *Oils and fats. vol. 2. Animal carcass fats* (pp. 77-122). Leatherhead, Surrey, UK: Leatherhead Publishing. ISBN 0905748743.

[28]. Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy*, *56*(8), 365-379.

[29]. Kris-Etherton, P. M., Harris, W. S., & Appel, L. J.; American Heart Association Nutrition Committee. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation, 106*(21), 2747-2757.

[30]. Chérif, J. K., Jémai, S., Rahal, N. B., Jrad, A., & Trabelsi-Ayadi, M. (2010). Study of antioxidant content and antiradical capacity of fresh and industrial waste of Tunisian tomato. Valorization of Tunisian tomato in bioactive molecules. *Tunisian Journal of Medicinal Plants and Natural Products*, *4*, 116-125.

[31]. Slimestad, R., & Verheul, M. J. (2009). Review of flavonoids and other phenolics from fruits of different tomato (*Lycopersicon esculentum Mill.*) cultivars. *Journal of the Science of Food and Agriculture, 89*, 1255-1270.

[32]. Zeb, A. (2004a). Chemical and nutritional constituents of sea buckthorn juice. *Pakistan Journal of Nutrition*, *3*(2), 99-106.

[33]. Suryakumar, G., & Gupta, A. (2011). Medicinal and therapeutic potential of sea buckthorn (*Hippophae rhamnoides L.*). *Journal of Ethnopharmacology*, *138*(2), 268-278.

[34]. Zadernowski, R., Naczk, M., & Amarowicz, R. (2003). Tocopherols in sea buckthorn (*Hippophae rhamnoides L*.) berry oil. *Journal of the American Oil Chemists' Society*, *80*(1), 55-58.

[35]. Eccleston, C., Baoru, Y., Tahvonen, R., Kallio, H., Rimbach, G. H., & Minihane, A. M. (2002). Effects of an antioxidant-rich juice (sea buckthorn) on risk factors for coronary heart disease in humans. *The Journal of Nutritional Biochemistry*, *13*(6), 346-354.

[36]. Dulf, F. V. (2012). Fatty acids in berry lipids of six sea buckthorn (*Hippophae rhamnoides L.*, subspecies carpatica) cultivars grown in Romania. *Chemistry Central Journal*, *6*(1), 106.

[37]. Rösch, D., Bergmann, M., Knorr, D., & Kroh, L. W. (2003). Structure − antioxidant efficiency relationships of phenolic compounds and their contribution to the antioxidant activity of sea buckthorn juice. *Journal of Agricultural and Food Chemistry*, *51*(15), 4233-4239.

[38]. Radenkovs, V., Püssa, T., Juhnevica-Radenkova, K., Anton, D., & Seglina, D. (2018). Phytochemical characterization and antimicrobial evaluation of young leaf/shoot and press cake extracts from *Hippophae rhamnoides L*. *Food Bioscience*, *24*, 56-66.

[39]. Li, T. S., Beveridge, T. H., & Oomah, B. D. (2003). Nutritional and medicinal values. *Sea buckthorn (Hippophaė rhamnoides L.): Production and utilization*, 101-108.

[40]. Nuernberg, K., Nuernberg, G., Priepke, A., & Dannenberger, D. (2015). Sea buckthorn pomace supplementation in the finishing diets of pigs-are there effects on meat quality and muscle fatty acids? *Archiv fuer Tierzucht*, *58*(1), 107.

[41]. Baum, S. J., Kris-Etherton, P. M., Willett, W. C., Lichtenstein, A. H., Rudel, L. L., Maki, K. C., ... & Block, R. C. (2012). Fatty acids in cardiovascular health and disease: a comprehensive update. *Journal of Clinical Lipidology*, *6*(3), 216-234.

[42]. Miura, K., Stamler, J., Brown, I. J., Ueshima, H., Nakagawa, H., Sakurai, M., ... & Curb, J. D. (2013). Relationship of dietary monounsaturated fatty acids to blood pressure: the International Study of Macro/Micronutrients and Blood Pressure. *Journal of Hypertension*, *31*(6), 1144.

[43]. Arif, S., Ahmed, S. D., Shah, A. H., Hassan, L., Awan, S. I., Hamid, A., & Batool, F. (2010). Determination of optimum harvesting time for Vitamin C, oil and mineral elements in berries sea buckthorn (*Hippophae rhamnoides*). *Pakistan Journal of Botany*, *42*(5), 3561-3568.

[44]. Bal, L. M., Meda, V., Naik, S. N., & Satya, S. (2011). Sea buckthorn berries: A potential source of valuable nutrients for nutraceuticals and cosmoceuticals. *Food Research International*, *44*(7), 1718-1727.

[45]. Sabir, S. M., Maqsood, H., Ahmed, S. D., Shah, A. H., & Khan, M. Q. (2005). Chemical and nutritional constituents of sea buckthorn (*Hippophae rhamnoides* ssp. *turkestanica*) berries from Pakistan. *Italian Journal of Food Science 17*, 455-462.

[46]. Teleszko, M., Wojdyło, A., Rudzińska, M., Oszmiański, J., & Golis, T. (2015). Analysis of lipophilic and hydrophilic bioactive compounds content in sea buckthorn (*Hippophae rhamnoides L*.) berries. *Journal of Agricultural and Food Chemistry*, *63*(16), 4120-4129.

[47]. Gao, X., Ohlander, M., Jeppsson, N., Björk, L., & Trajkovski, V. (2000). Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn

(*Hippophae rhamnoides L.*) during maturation. *Journal of Agricultural and Food Chemistry*, *48*(5), 1485-1490.

[48]. Benakmoum, A., Abbeddou, S., Ammouche, A., Kefalas, P., & Gerasopoulos, D. (2008). Valorisation of low quality edible oil with tomato peel waste. *Food Chemistry*, *110*(3), 684-690.

[49]. Krinsky, N. I. (1993). Actions of carotenoids in biological systems. *Annual Review of Nutrition*, *13*(1), 561-587.

[50]. Shixian, Q., Dai, Y., Kakuda, Y., Shi, J., Mittal, G., Yeung, D., & Jiang, Y. (2005). Synergistic anti-oxidative effects of lycopene with other bioactive compounds. *Food Reviews International*, *21*(3), 295-311.

[51]. Lalas, S., Gortzi, O., Tsaknis, J., & Sflomos, K. (2007). Irradiation effect on oxidative condition and tocopherol content of vegetable oils. *International Journal of Molecular Sciences*, *8*(6), 533-540.

[52]. Nour, V., Corbu, A. R., Rotaru, P., Karageorgou, I., & Lalas, S. (2018). Effect of carotenoids, extracted from dry tomato waste, on the stability and characteristics of various vegetable oils. *Grasas y Aceites*, 69(1), e238.

[53]. Applegate, E. (2000). Introduction: nutritional and functional roles of eggs in the diet. *Journal of the American College of Nutrition*, *19*(sup5), 495S-498S.

[54]. Chung, H. Y., Rasmussen, H. M., & Johnson, E. J. (2004). Lutein bioavailability is higher from lutein-enriched eggs than from supplements and spinach in men. *The Journal of Nutrition*, *134*(8), 1887-1893.

[55]. Sahin, N., Akdemir, F., Orhan, C., Kucuk, O., Hayirli, A., & Sahin, K. (2008). Lycopene-enriched quail egg as functional food for humans. *Food Research International*, *41*(3), 295-300.

[56]. Akdemir, F., Orhan, C., Sahin, N., Sahin, K., & Hayirli, A. (2012). Tomato powder in laying hen diets: effects on concentrations of yolk carotenoids and lipid peroxidation. *British Poultry Science*, *53*(5), 675-680.

[57]. Hencken, H. (1992). Chemical and physiological behavior of feed carotenoids and their effects on pigmentation. *Poultry Science*, *71*(4), 711-717.

[58]. Blount, J. D., Surai, P. F., Houston, D. C., & Møller, A. P. (2002). Patterns of yolk enrichment with dietary carotenoids in gulls: the roles of pigment acquisition and utilization. *Functional Ecology*, *16*(4), 445-453.

[59]. Englmaierová, M., Skrivan, M., & Bubancová, I. (2013). A comparison of lutein, spray-dried Chlorella, and synthetic carotenoids effects on yolk colour, oxidative stability, and reproductive performance of laying hens. *Czech Journal of Animal Science*, *58*(9), 412-419.

[60]. Benakmoum, A., Larid, R., & Zidani, S. (2013, January). Enriching egg yolk with carotenoids & phenols. In *Proceedings of World Academy of Science, Engineering and Technology* (No. 79, p. 172). World Academy of Science, Engineering and Technology (WASET).

[61]. Karadas, F., Grammenidis, E., Surai, P. F., Acamovic, T., & Sparks, N. H. C. (2006). Effects of carotenoids from lucerne, marigold and tomato on egg yolk pigmentation and carotenoid composition. *British Poultry Science*, *47*(5), 561-566.

[62]. Corbu, A.R., Nour, V., 2017. Development and evaluation of a reverse-phase HPLC method for the analysis of carotenoids in egg yolk. *Ann. Univ. Craiova, Biol. Hort. Food Tech. Env. Eng. Ser. 22*, 81-88.

[63]. Alagawany, M., Farag, M. R., Dhama, K., & Patra, A. (2018). Nutritional significance and health benefits of designer eggs. *World's Poultry Science Journal*, *74*(2), 317-330.

[64]. Surai, P. F., & Sparks, N. H. C. (2001). Designer eggs: from improvement of egg composition to functional food. *Trends in Food Science & Technology*, *12*(1), 7-16.

[65]. Galobart, J., Barroeta, A. C., Baucells, M. D., Cortinas, L., & Guardiola, F. (2001). α-Tocopherol transfer efficiency and lipid oxidation in fresh and spray-dried eggs enriched with ω 3-polyunsaturated fatty acids. *Poultry Science*, *80*(10), 1496-1505.

[66]. Meluzzi, A., Sirri, F., Manfreda, G., Tallarico, N., & Franchini, A. (2000). Effects of dietary vitamin E on the quality of table eggs enriched with n-3 long-chain fatty acids. *Poultry Science*, *79*(4), 539-545.

[67]. Young, A. J., & Lowe, G. M. (2001). Antioxidant and prooxidant properties of carotenoids. *Archives of Biochemistry and Biophysics*, *385*(1), 20-27.

[68]. Galobart, J., Barroeta, A. C., Baucells, M. D., & Guardiola, F. (2001). Lipid oxidation in fresh and spray-dried eggs enriched with ω3 and ω6 polyunsaturated fatty acids during storage as affected by dietary vitamin E and canthaxanthin supplementation. *Poultry Science*, *80*(3), 327-337.

[69]. Hung, Y. T., Hanson, A. R., Shurson, G. C., & Urriola, P. E. (2017). Peroxidized lipids reduce growth performance of poultry and swine: a meta-analysis. *Animal Feed Science and Technology*, *231*, 47-58.