

**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

An integrated approach to add value to acerola fruit (*Malpighia emarginata*): antioxidant extraction and its performance in food emulsion

Ana Carolina Loro

Thesis presented to obtain the degree of Doctor in Science.
Area: Food Science and Technology

**Piracicaba
2020**

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Agroindustrial Engineering

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Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP

Loro, Ana Carolina

An integrated approach to add value to acerola fruit (*Malpighia emarginata*):
antioxidant extraction and its performance in food emulsion / Ana Carolina Loro.
- - Piracicaba, 2020.

99 p.

Tese (Doutorado) - - USP / Escola Superior de Agricultura "Luiz de
Queiroz".

1. Acerola 2. Antioxidantes 3. Emulsões alimentícias 4. Oxidação 5.
Sensorial I. Título

DEDICATORY

Dedico esse trabalho de pesquisa primeiramente a Deus pois sem a minha fé e crença Nele não teria força e resiliência para seguir sozinha, aliás contei e conto com sua onipresença e onipotência em todos os momentos de minha vida. Dedico também a todos os meus familiares, amigos, colegas pesquisadores e professores com os quais tive o privilégio da convivência, amizade, colaboração e troca de experiências durante a pesquisa. Não poderia deixar de dedicar e mencionar os colegas e profissionais patrícios (de Porto, Portugal) com os quais convivi por quatro meses durante parte da realização do projeto. Pude desfrutar de ciência, cultura e experiência de vida com esse curto, mas proveitoso e excelente, período de intercambio na Faculdade de Ciências da Universidade do Porto (FCUP) e na Sense Test, empresa que gentilmente me acolheu e permitiu a realização do estágio/intercambio, que acho mais apropriado ser chamado de “Experiência Vivencial”.

Dedico a todos que estiveram ao meu lado durante essa parte da minha trajetória tanto de vida quanto acadêmica. Acredito que a pesquisa, cultura e educação devem estar alinhadas e lado a lado pois são os pilares da formação como pessoas e seres humanos que somos e muitos dizem e formam nossos valores morais e éticos.

A trajetória não é e não foi fácil, mas quando se tem respeito, colaboração e empatia de pessoas que estão em seu convívio social, ela se torna um pouco mais fácil e menos custosa física e mentalmente. Não acredito que podemos passar por todos os obstáculos sem a convivência social e familiar. Quando se atravessa a ponte com alguém altruísta, confiante e positiva, ou com alguém com uma visão e mente diferentes da sua, em resumo, com pessoas que lhe passem segurança e boas energias, temos mais força e coragem. Juntos podemos ir mais longe.

A dedicatória me permitiu uma reflexão, aliás diante de tantos textos e parágrafos escritos até agora, uma conclusão que pude tirar é que aprendi a me conhecer melhor e a conhecer e entender as pessoas ao meu redor com um outro olhar. Ultimamente, principalmente meus familiares acho que vão concordar com essa proposição que fiz acima e adicionalmente se pudessem ler essa dedicatória e me dizer algo, certamente me diriam que estou diferente e estou em constante mudança, o que acho extremamente benéfico e saudável para o corpo e mente, afinal estar em constante mudança e sempre à disposição e aberto a aprender e colaborar com os demais é muito bom e nos faz crescer quer seja como pessoa quer seja

como profissional quer seja como indivíduo inserido na sociedade. Outra coisa que claramente me diriam é que estou mais falante, reflexiva e preparada para novas experiências.

Depois dessa épica dedicatória reflexiva e extremamente sentimental (quase uma epopeia ou um teatro grego), sim sou muito emotiva, ainda gostaria de mencionar o nome de quatro pessoas, em especial, que se não fosse por elas e pelo amor incondicional e recíproco delas por mim, talvez esse sonho não se concretizasse ou talvez eu atravessasse a ponte de forma mais lenta e gradual e de forma mais dolorida. Dedico esse trabalho as minhas avós, Irene e Magali, as quais são razão de minha existência e me ensinaram o que é amor. Pude retribuir um pouquinho do amor delas em duas marcas em meu corpo (minhas tatuagens), pois não quero de forma alguma e em nenhum minuto de minha existência esquecer do amor delas! Amo vocês incondicionalmente. Não poderia esquecer dos meus heróis maiores, meus pais, Lízia e Marcelo, com os quais muito aprendi e aprendo até hoje. Vocês são meus exemplos.

Mesmo não citando anteriormente, minhas irmãs Ana Paula e Ana Beatriz, são pessoas que admiro e não poderia deixar de dedicar a elas o fruto do meu trabalho, a concretização de um de meus sonhos, a vida acadêmica e a pesquisa! Acredito que a escola, ainda é o lugar de formar “gente” e que a profissão de professor é a mais bela que existe. Com essas duas menininhas, muito fortes e decididas, pude também aprender e compreender o que é amor e com elas, com certeza, a travessia da ponte, aquela que mencionei não sei quantas vezes nessa dedicatória, foi bem mais fácil.

Sou muito grata e dedico a todos meus amigos, colegas e familiares esse trabalho.

ACKNOWLEDGMENTS

Depois de uma dedicatória digna de teatro grego, ou de uma peça de William Shakespeare. Só tenho a agradecer e o sentimento é de gratidão por tudo e todos. Não gosto de citar nomes para não esquecer ninguém! Agradeço a minha família, meu pilar. Agradeço também aos antigos amigos, novos amigos que fiz.

Agradeço aos meus queridos pais, Lízia e Marcelo, por todo amor, suporte e carinho. Vocês são tudo em minha vida, meus exemplos. Me ajudam e me ajudaram a passar todos os obstáculos sempre juntos comigo. Mais que tudo, agradeço a minhas avós Irene e Magali. Vocês são o que de mais especial tenho na vida, sem vocês nada disso seria possível. E se hoje sou a pessoa que sou, com minhas convicções e com minhas verdades devo isso muito a vocês! Deus me presenteou de uma forma inexplicável ao me dar duas avós tão especiais.

Agradeço ainda a todos os meus amigos e colegas, vocês tornaram a trajetória mais leve. Obrigada por tudo. Aqui cabe um agradecemos a Profa Thais Vieira, minha orientadora. Obrigada por tudo, pela confiança depositada em mim, pelos ensinamentos, pelo convívio. Enfim obrigada por ser uma orientadora, uma amiga, uma excelente professora. Enfim sou grata por tudo que fez por mim tanto no lado profissional quanto pessoal. Admiro sua força e o poder que você tem de dar conta de tudo, passar por tudo e sempre ter uma palavra de confiança que acalma seus orientados, quando estão em momentos difíceis ou com problemas.

Por fim agradeço aos colegas de Portugal, que citei na dedicatória. Vocês fazem parte da minha vida. Trouxe um pouquinho de todos comigo e de tudo que me ensinaram. Obrigada por tudo.

Enfim, agradeço a todos que estiveram ao meu lado e me ajudaram na concretização de mais esse sonho.

EPIGRAPH

“É pela educação, muito mais do que pela instrução, que vamos atingir o progresso moral. A educação, se bem compreendida, é a chave do progresso moral”

Allan Kardec

“Nossas dúvidas são traidoras e nos fazem perder o que, com frequência, poderíamos ganhar, por simples medo de arriscar. O débil, acovardado, indeciso e servil não conhece, nem pode nhecer o generoso impulso que guia aquele que confia em si mesmo, e cujo prazer não é de ter conseguido a vitória, se não de sentir capaz de conquista-la”

William Shakespeare

“O grande inimigo do conhecimento não é a ignorância, é a ilusão de ter o conhecimento”

Stephen Hawking

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RESUMO

Uma abordagem integrada para agregar valor ao fruto de acerola (*Malpighia emarginata*): extração de antioxidantes e seu desempenho em emulsão lipídica

Compostos antioxidantes têm a capacidade de proteger, prevenir ou reduzir os danos causados pelos fenômenos da oxidação causados por radicais livres. São de grande importância nos mecanismos de defesa das plantas e outros sistemas biológicos, atuando diretamente nas etapas de geração e propagação de radicais livres do processo oxidativo, tanto nos alimentos quanto nos sistemas biológicos. Em alimentos, atuam prevenindo e retardando os processos peroxidação lipídica, autooxidação e desenvolvimento de rancidez, por meio de neutralização dos radicais livres, desencadeadores destas reações que levam ao desenvolvimento de sabores e compostos químicos indesejáveis, além da redução da qualidade e da vida útil dos alimentos. Nesse sentido o uso de compostos antioxidantes é uma necessidade da indústria de alimentos, que normalmente utiliza compostos sintéticos. O questionamento sobre a inocuidade destes e a recente demanda por ingredientes naturais pelos consumidores têm motivado estudos de fontes alternativas de antioxidantes. Grande atenção tem sido dada ao ácido ascórbico, tocoferóis e carotenoides, substâncias que apresentam grande potencial de aplicação industrial. Nessa linha de pesquisa, tem-se nos frutos de acerola (*Malpighia emarginata* DC.) Uma importante fonte de antioxidantes naturais, devido aos elevados teores de ácido ascórbico e compostos fenólicos. O estudo teve por objetivo aplicação de extratos obtidos da acerola como substituto aos antioxidantes sintéticos em matrizes lipídicas e foi realizado em três etapas: estudo das condições de obtenção dos extratos utilizando técnicas de planejamento experimental e otimização; estudo da estabilidade oxidativa de sistemas lipídicos comparando extratos naturais com antioxidantes sintéticos; avaliação sensorial de emulsões adicionadas de extratos naturais. Os resultados evidenciaram a elevada capacidade antioxidante dos extratos obtidos em condições otimizadas e sua significativa ação antioxidante na estabilidade oxidativa de sistemas lipídicos. Observou-se elevada aceitação sensorial e baixa diferenciação de perfil sensorial de emulsões adicionadas de extratos naturais em comparação aos sintéticos. Conclui-se que os antioxidantes à base de acerola são uma alternativa para substituição aos antioxidantes sintéticos em alimentos de base lipídica pela indústria de alimentos, sem acarretar alterações sensoriais.

Palavras-chave: Acerola, Antioxidantes, Emulsões alimentícias, Oxidação, Sensorial

ABSTRACT

An integrated approach to add value to acerola fruit (*Malphigia emarginata*): antioxidant extraction and its performance in food emulsion

Antioxidants have the ability to protect, prevent, or reduce the damage caused by the phenomena of oxidation due to free radicals' activity. They play a key role in the defense mechanisms of plants and other biological systems, acting directly in the early stages of generation and propagation of free radicals in the oxidative process, in both food and biological systems. In foods, they act by preventing and/or delaying the processes of lipid autoxidation through the neutralization of free radicals. Autoxidation lead to the development of undesirable flavors and chemical compounds in food, besides deterioration of quality and shortening the shelf life of food products. The use of antioxidant compounds is a necessity in the food industry, which normally uses synthetic products. The questioning of the innocuity of synthetic antioxidants and the recent demand for natural ingredients by consumers have motivated the study of alternative sources and their processing parameters for food application. Attention has been given to ascorbic acid, tocopherols, and carotenoids that present known potential for food application. Acerola fruits (*Malphigia emarginata* DC.) are an important source of natural antioxidants, due to the high levels of ascorbic acid and phenolic compounds. In this work natural extracts obtained from acerola pulp and seeds were applied in lipid matrices. The study consisted of three stages: extraction conditions determination using experimental design and optimization methods; oxidative stability study in lipid system models comparing natural extracts to synthetic ones; and sensorial evaluation of emulsions added off natural antioxidants. The results evidenced a high antioxidant capacity of extracts obtained under optimized conditions and a significant antioxidant action in the oxidative stability in lipid systems. In addition, it was observed high sensorial acceptance and low sensorial profile differentiation of emulsions added of natural extracts in comparison to the synthetic antioxidants. Therefore, acerola is an efficient substitute for synthetic antioxidants in lipid-based foods without causing sensorial alteration.

Keywords: Acerola, Antioxidants, Food emulsions, Oxidation, Sensory analysis

INTRODUCTION

An antioxidant compound is generally defined as a class of heterogeneous molecules that, present in low concentrations, are able to reduce or protect a system against the damage caused by oxidative stress, which is caused by free radicals (HALLIWELL, 1990; HALLIWELL; GUTTERIDGE, 2007). Natural antioxidants act in the defense mechanisms of animal and plants, playing a direct role in the oxidative process, basically in the stages of generation and propagation of free radicals, both in food and in biological systems (ESPIN et al., 2000; SUJA; JAYALEKSHMY; ARUMUGHAN, 2004). In living beings (animals or plants), free radicals attack essential biological molecules, leading to many degenerative diseases such as cancer and atherosclerosis. In food, free radicals are responsible for the autoxidation process, lipid peroxidation and rancidity development, reactions that lead to the development of strange and undesirable flavors and chemical compounds in foods (LABUZA, 1971; SHAHIDI; JANITHA; WANASUNDARA, 1992; ANGELO, 1996).

Nowadays there is a strong scientific questioning about the innocuity and food safety of the use of additives and synthetic preservatives by the food industry, evidenced by the researches that have not comproved the safety of these substances for human health and by the recent demand for healthier and natural products by the consumers, has motivated studies in the food technology area. Research on substances extracted from natural sources that have significant antioxidant action have become of importance and the current needs are: identification of new additives and technology coadjuvants that have effective and significant antioxidant potential, which can be proven by the methods of evaluation of the antioxidant capacity; development of new technologies and extraction processes of these compounds; assessment of industrial viability for food application; the use of raw vegetable materials little explored commercially and industrially.

The major challenge for the industry consists, therefore, in finding naturals alternatives and substitutes to the known and widely used synthetic antioxidants, taking into account that the main purpose of using these substances is to conserve and preserve the nutritional and sensorial quality of the products, aiming extend their shelf life. Synthetic antioxidants are widely used in lipid-based food products in order to avoid and delay the lipid oxidation of the fatty acids present. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate, tert-butyl hydroquinone (TBHQ) and sorbates (2,4-hexadienoates) are the most commonly used.

In order to attend the current trends, great attention has been given to substances such as ascorbic acid, tocopherols, tocotrienols, and carotenoids (betacarotene), since these compounds show potential for industrial application as they improve the stability and shelf life of food products (NOGUCHI; NIKKI, 2000). In addition, there is an advantage from the economic point of view, because if the antioxidant extraction source is a non-value-added raw material, such as peels and seeds generated in the fruit processing, it represents socio-environmental advantage compared to the synthetic compounds. In this research area for the substitution of synthetic substances in the food industry, acerola fruits, also known as Antilles cherry (*Malpighia emarginata* DC.), represent a satisfactory and potential alternative due to its composition in natural antioxidants. Native from Antilles, acerola was propagated over whole South America, including in Brazil, due to the good

adaptation to the soil and climate (VENDRAMINI, TRUGO, 2000). Acerola fruits present a higher content of ascorbic acid (vitamin C) and other nutrients such as carotenoids, thiamine, riboflavin, niacin, and minerals, such as calcium and phosphorus (DE ASSIS et al., 2001; VENDRAMINI, TRUGO, 2000).

This work presents a review on antioxidants, their mechanisms of action, industrial uses, focusing on natural antioxidants extracted from fruits and vegetables (Chapter 1). The experimental study was developed on the application acerola fruits extracts (from pulp and seeds) in a based-lipid matrix (mayonnaise), as a substitute to synthetic antioxidants. The processes conditions to produce natural extracts from acerola pulp and seeds were studied by means of experimental design and optimization methodology. The antioxidant activity optimized extracts and their performance on oxidative stability essays (in different emulsified lipid systems model) compared to synthetic BHA and BHT were evaluated (Chapter 2). Finally, Chapter 3 presents a sensorial evaluation of mayonnaise type emulsion added off natural extracts and synthetic antioxidants was carried out, through a sensory acceptance test and a flash profile sensory description test, which promotes a description of the samples by means of attribute surveys.

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CHAPTER 1. ANTIOXIDANTS AND LIPID OXIDATION IN BIOLOGICAL SYSTEMS AND FOODS – A REVIEW OF NATURAL SOURCES AND OXIDATION ASSESMENT

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Review paper submitted to “European Food Research and Technology”

Abstract:

Synthetic substances such as TBHQ, BHA, and BHT have satisfactory and comproved antioxidant activity in fats and oils and also in lipid-based foods. However, the problem of using these products related to innocuity to human health has been rising for decades. Some experiments have demonstrated that these substances are not completely innocuous as expected. Due to this, many studies were developed with the aim of finding natural substitutes from vegetable sources that perform similar antioxidant activity. Acerola fruit is a potential alternative, since it presents a considerable range of compounds with antioxidant action, whose major representative is ascorbic acid (vitamin C). This review presents the main natural antioxidants as well as their sources, focusing on the evaluation methods and antioxidants mechanisms to preserve and avoid chain reactions that promote lipid oxidation in biological systems and foods, with an emphasis on acerola fruits.

Keywords: *Malphigia emarginata* DC; Natural extracts; Antioxidant activity; Lipid oxidation; Shelf-life

Introduction

The main cause of deterioration and loss of nutritional quality in lipid-based foods (emulsions for example), oils and fats is the lipid oxidation of their mono and polyunsaturated fatty acids, caused mainly by free radicals. The main catalytic agents are light, oxygen, and the presence of transition metals. The industry has been using synthetic antioxidants to reduce and/or delay the oxidative process (a set of oxidation reactions that occur in fatty acids). However, the use of synthetic substances has been questioned and better studied, since toxicological studies with animals have shown that known and widely used products (such as TBHQ, BHA, and BHT) can present carcinogenic and other undesirable effects to human health, which explains the prohibition of the use of these substances by several countries [1-2]. For example, Europe and Canada have banned the use of TBHQ [3]. In Brazil, the use of these additives in food is controlled by Health Ministry, which limits the use to 200 mg kg⁻¹ of oil for BHA and TBHQ, and 100 mg kg⁻¹ of oil for BHT [4] Among the synthetic compounds with significant antioxidant activity, TBHQ is considered the most effective, since it presents high stability to heating, and is widely used in bulk oils [5].

Considering these questions raised about the innocuity of application of synthetic antioxidants in foods, it was necessary to identify and isolate natural antioxidants that have the same functional

properties and act as substitutes for synthetics in prevention of oxidative deterioration of foods. Among the innumerable sources of natural antioxidants, there are cereals, seeds, and peels of fruits and vegetables (fruit and vegetable industrial residues in general), mushrooms, herbs and spices and medicinal plants [6-10]. In this context, there is a great potential in acerola fruit as antioxidant source for industrial due to its high antioxidant capacity.

The objective of this work presents a review about lipid oxidation, the main cause of undesirable lipid alteration, as well as the methods for shelf life evaluation. It is also presented the main natural antioxidant compounds, their sources, and their use as an alternative to synthetics, with an emphasis on the acerola fruit antioxidants compounds (*Malpighia emarginata* DC).

1. Antioxidant compounds

Antioxidants represent a broad class of compounds that step in oxidative processes, including in degradation of food nutrients and essential biological molecules [11-12]. Recently, with growing concern about determining which antioxidants are safe for human health, the choice is usually for natural antioxidants, especially plant-derived antioxidants.

The main class of antioxidant compounds found in natural sources are phenolic (flavonoids or non-flavonoids), which have as one of health benefits, for example, the inhibition/reduction of oxidation of low-density lipoprotein (LDL cholesterol) [13-18]. To accomplish its protective function, antioxidants present some mechanisms of action, such as free radical scavengers, metal chelating agents and singlet oxygen sequestrants [19]. It is important to emphasize that the low consumption of fruits and vegetables, natural sources of these substances with antioxidant activity, is associated with the development of stress-related disorders and other diseases [13; 20-22]

They can be classified as primary antioxidants, which have the ability to interrupt chain reactions of free radicals, acting primarily as radical scavengers and/or electron or hydrogen donors to free radicals, and for this one of the mechanisms of action involves yielding electrons/hydrogen to a free lipid radical so that it takes on a thermodynamically more stable form. For example, some phenolic compounds have electron donor groups in the orto and para positions of their cyclic chain. Antioxidants can also be classified as secondary (also called processing antioxidants), which have organophosphite groups and thioesters in their structure and are known mainly for reducing the initiation of autoxidation process, and for that they present some mechanisms of action such as decomposition of peroxide radicals (hydroperoxides), metal complexation, absorption of ultraviolet radiation and scavenging or deactivation of singlet oxygen. The main examples are ethylenediaminetetraacetic acid (EDTA) and citric acid [18-19; 23-27].

Also related to the classification, antioxidants can be endogenous (that are part of the body's defense system) whose main examples are uric acid, bilirubin, albumin, metallothioneins, as well as the enzymes superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-s-transferase. They may also be exogenous, which can be naturals, obtained by the diet, like vitamin C, vitamin E, carotenoids, phenolic compounds such as flavonoids, phenolic acids, anthocyanins, proteins such as transferrin, albumin, and some minerals such as selenium, or synthetic

such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), gallates and others. It is recognized that vegetables, fruits, grains and beverages such as tea, juice and wine are significant sources of natural exogenous antioxidants [18; 28-29].

1.1 Phenolic compounds

The phenolic compounds represent a wide variety of phytochemicals, which are derived from phenylalanine and tyrosine and are chemically characterized by the presence of one or more aromatic rings linked to at least one hydroxyl radical and/or other substitutes [27; 30]. They can be divided according to the number of phenolic rings and with the structures to which they are linked [31-32]. The groups of phenolic compounds more abundant in foods are the flavonoids, phenolic acids, simple phenols, tannins, lignins, coumarins and tocopherols [32-35]. It is known that dietary polyphenols are beneficial to human health, exercising a range of biological effects, as elimination and/or scavenging of free radicals, metals chelators, enzyme activity modulator and change pathways of signal transduction [36].

Not always the most common in foods are the most biologically active, and this occurs for different reasons as intrinsic low activity, lower intestinal absorption or fast metabolization and excretion [37]. The main sources of phenolic compounds are the citrus fruits such as lemon, orange and mandarin, as well as other fruits such as cherry, grape, plum, pear, apple and papaya, being found in greater quantities in pulp compared with fruit juice. Green pepper, broccoli, purple cabbage, onions, garlic and tomatoes are also excellent sources of these compounds [38]. In vegetables are essential in the plant growth and reproduction, as well as act as antipathogenic agent and contribute to the color or pigmentation [35]. In food are responsible for the color, astringency, flavor [39] and oxidative stability [40].

Bioactive compounds that include phenolics are found in vegetables in free form or linked/complexed to sugars (glycosylated) and/or proteins [41]. Phenolics compounds are since simple to high degree of polymerization molecules with variable structure. Ribéreau-Gayon [42] proposal consists in a classification of them in three categories: little distributed in nature, polymers and widely distributed in nature. Within this classification in the category of phenolic little distributed in nature, it is possible found a reduced number, although with a certain frequency, simple phenols such as pyrocatechol, hydroquinone, resorcinol, and aldehydes derived from benzoic acids, which are constituents of essential oils, such as vanillin [43]. In the category of polymers some free phenolic are found (tannins and the lignans). The last category of compounds widely distributed in nature includes the most commonly phenolic of the vegetables: the flavonoids (anthocyanins, flavonols, and their derivatives), the phenolic acids (cinnamic and benzoic acids and their derivatives), and the coumarins [44].

According to the mode of action, phenolic compounds are included in the category of primary antioxidants as eliminators of free radicals, being very effective in autoxidation prevention [45]. Interact preferentially with the peroxy radical for being this most prevalent in the autoxidation phenomenon and have less energy than other radicals, which favors your hydrogen abstraction [46]. The resultant

fenoxil radical, although relatively stable, can interfere in propagation reaction when react with a peroxy radical, via interaction between radicals. The compound formed, by the ultraviolet light action and high temperatures, may lead to new radicals, compromising antioxidant efficiency, which is determined by the functional groups, and position that they occupy in the aromatic ring, as well as, by the size chain of these groups [45; 47].

1.2 Phenolic acids

The polyphenols or phenolic acids commonly found in plants are hydroxy derived from cinnamic and benzoic acid, that presented carboxyl functional group in your structure [30; 48-49]. They are classified into two groups: hydroxycinnamic acid derivatives and hydroxybenzoic acid derivatives. Hydroxycinnamic acid derivatives have an aromatic ring with a carbonic chain constituted of three carbons bonded to the aromatic ring. The p-coumaric, ferulic, caffeic, and synapic acids are common examples typically presented in the form of ester. The most common example is the chlorogenic acid, which is the quinic acid esterified to caffeic acid. They are also found in glycosylated form (linked to glycosides/sugars or complex carbohydrates), in protein-bound, in other cell wall polymers, and rarely as free phenolic acids form [50-52].

Chlorogenic acids are a class of phenolic esters (esterified phenolic acids) thermolabile that have beneficial properties to health as hepatoprotective, hypoglycemic and antiviral [53-55], being formed by some hydroxycinnamic acids (caffeic, ferulic and p-coumaric acid) and quinic acid [56]. The last phenolic acid cited, when linked with the caffeic acid, forms a phenolic ester called chlorogenic acid [57].

Chlorogenic acid (5-caffeolquinic acid) is the main biologically active dietary phenol of foods. When hydrolyzed by the intestinal microflora it produces various aromatic acids as metabolites, including caffeic and quinic acids [58]. As well as caffeic acid, chlorogenic acid presented adjacent/free hydroxyl groups linked in the carbon of the aromatic ring, and this is related to the benefits (antimutagenic, anticarcinogenic, and mainly antioxidant *in vitro* activity) [59]. Coffee and legumes (beans) are the main sources of chlorogenic acid in the diet [60].

Ferulic acid is another phenolic acid widely found in vegetables, fruits and beverages, such as coffee and beer. It is derived from caffeic acid and belongs to the hydroxycinnamic acids class [32; 61]. The interest in this and other phenolic acid derivatives from the caffeic acid began in late 1950, when Preziosi, Loscalzo and Bianchi [62] and Preziosi and Loscalzo [63-65] elucidated and developed the mechanisms of action of this substance in the human body and verified action related to bile secretion by the liver (coleretic) and decrease of serum lipid levels (hypolipidemic) in addition to diuretic functions. Even with the discovery of these and other beneficial functions to human body, only recently this and other phenolics gained attention as potential coadjuvants in therapies and treatments of various diseases induced by free radicals. Ferulic acid, in particular, presented as the new antioxidant compound with a strong cytoprotector activity, both the ability of eliminating free radicals as activating the cellular stress response. Some unfavorable points are related to pharmacokinetics and metabolic mechanisms/pathways of this, due its low bioavailability of this compound after

ingestion and/or oral administration and the limited number of clinical studies conducted in order to verify and demonstrate its efficacy and pharmacologic, toxicology safety, which limited the evidence regarding the potential interest of phenolic acid in relation to your action on the human organism [66].

P-coumaric acid other phenolic acids are widely distributed in fruits (pear, apple, grape), cereals, legumes (beans), and other vegetables (potato, tomato and teas). P-coumaric acid is a metabolite present in plants, being intermediate product in the synthesis of other phenols [60] and participating in the metabolic pathways of the phenylpropanoid. These substances are plants metabolites, components of plant essential oils that have as basic chemical structure a group ring phenyl (benzene ring) attached to a side chain with three carbon atoms [67]. Studies with animals (*in vivo*) suggest that p-coumaric acid presents antioxidant and anti-inflammatory properties in the mucosa of intestinal cells in rats [68-74]. It has also been attributed to this phenolic acid the ability to prevent the LDL cholesterol oxidation (a low-density lipoprotein) avoiding disruption of the chain caused by reactive species (free radicals) [70], preventing lipid peroxidation on the basis in its synergistic antioxidant activity with other natural antioxidant compound named tocopherol (vitamin E) [72]. Its mechanism of action is not fully known; However, it has been proposed that its antioxidant action is based on the capture/scavenger of reactive oxygen species (ROS) [75].

As well as the phenolic acids mentioned above, we have representatives of the class of flavonoids (another class of phenolic compounds) such as quercetin, rutin, catechins and epicatechin, which also are widely distributed in plants in nature and has great importance as antioxidants [13; 58; 60; 76-79].

1.3 Flavonoids

Flavones and related compounds occur widely in vegetables. Catechins, quercetin, and pyrocatechol derivatives flavonoids have high antioxidant activity and have been used to stabilize lard. Polyhydroxychalcon are powerful antioxidants found in cabbage and other leafy vegetables, peppers, soy, peas, peanuts, cocoa, cottonseed and many other vegetables. Some flavonoids derivatives of pyrogallol, as the tannins in tea, also have antioxidant activity. The flavonoids of the tea are of practical interest, because tea presents sediment and leaves that are not suitable for drying and processing, and therefore these "waste" would be available in significant quantities for the preparation of antioxidants extracts [80-81].

Flavonoids are formed in plants from aromatic aminoacids phenylalanine and tyrosine, and malonate [82]. The basic structure of flavonoids is the flavan ring, which consists of 15 carbon atoms arranged in three rings (C6-C3-C6), A, B and C (Figure 1). The flavonoids classes differ in the level of oxidation and substitution pattern of the C ring, while the individual compounds within the class differ in the substitution pattern of rings A and B [83].

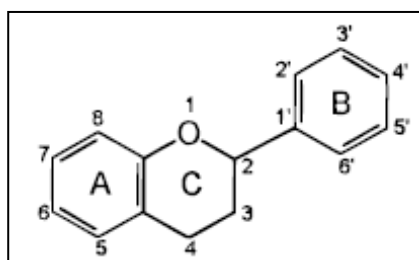


Figure 1. Flavonoid basic chemical structure

They are compounds that usually occur in plants in the form of glycosylated derivatives, which contribute to the intensity color of the different shades of blue, red and orange in leaves, flowers and fruits [84]. In addition to vegetables and fruits, the flavonoids are found in seeds, nuts, grains, spices, medicinal plants, and also in beverages such as wine (particularly red wine), teas, and, at lower levels, in beer [85]. They play different roles in the ecology of plants, being one of them like a visual pigment for pollinating insects, due to the fact the flavones, flavonols and anthocyanidins exhibit attractive color and being considerate natural pigments of plants and therefore helpers in pollination process. Another important role played by flavonoids is in defense mechanisms of the plant, since the catechins and other flavanols presented certain astringency, which can represent a system of defense against insect's attack [86].

In addition to these functions, the class of flavonoids, they still act in the photosynthesis process as catalysts of the light phase, as regulators of iron chains involved in phosphorylation [87], as protectors of stress in plant cells for elimination of reactive oxygen species (ROS) produced by electron transport in photosynthetic system [88], and finally, due to its properties of absorption of UV radiation, they protect plants from solar UV radiation and reactive species (ROS) generated by UV radiation [89].

More specifically, the flavones apigenin and luteolin are common in cereal grains and herbs (parsley, rosemary, thyme), while its analogues hydrogenated hesperidin (glycosylated form of hesperitin) and naringenin are predominantly in citrus [90]. The flavonols quercetin and kaempferol are prevalent found in vegetable and fruit peels, with exception of the onions. Isoflavones are found most often in legumes, including soy, beans (black and green) and peas, alfalfa and sunflower seeds [91]. The flavan-3-ols (flavanols) such as catechins, epicatechin, epigallocatechin and their gallate esters are widely distributed in plants, although they are present in significant quantities in tea leaves. The flavans polymerized as proanthocyanidins (condensed tannins), that are dimers or oligomers of catechins and epicatechin, are present in apples, grapes, red berries (berries in general too), persimmon, black currant, sorghum and barley grains [92].

The anthocyanidins and its glycosylated forms (anthocyanins) are abundant in red fruits (berries) and red grapes [93]. Anthocyanins are substances that accumulate in the vacuoles of a large range of vegetative cells and tissues in reproductive organs of the plants [94]. They are also part of the plant pigments, as well as the carotenoids, being responsible for the coloration of plants (flowers,

leaves and fruit), ranging from red, pink, purple, until blue [95]. Structurally are formed by di or tri-hydroxy β -rings substituted containing a flavilium cation which, due to conjugated double bond absorbs light in the range of visible light, with the peak wavelength is around 500-550 nm. The range of derived anthocyanins are, as well as the colors that display in plants depends on the degree of hydroxylation and the number and/or type of replaced groups. Aglicons forms of anthocyanin, i.e., anthocyanidins, are usually penta or hexa-hydroxyl replaced and is not linked with organic sugars, proteins and phospholipids [96].

The functionality and performance mechanisms of these compounds have always been and remain questionable and require larger studies, this is due to the fact that the benefits derived from anthocyanins in vegetables are different depending on the species of the compound in question and the way in which he finds himself in the plant, causing a universal explanation about the mechanisms of action of anthocyanins is not possible [94]. Given this, the current search presents at least four recent evidence regarding the functionality of the anthocyanins, and they are: solar protection agent and antioxidant action, mediator of oxidative chain reaction induced by reactive oxygen species (ROS) [94; 97], metal chelators [98-104] and leaf senescence retarders, especially in plants that grow with nutrient deficiency [105-107].

2. Acerola and its antioxidants compounds

Acerola (*Malpighia emarginata* DC), a tropical fruit, is a small cherry with juicy pulp and sweet flavor, known primarily for its high content of vitamin C. The fruit origins from species native from the West Indies, which have adapted to Central and South America [108]. In the past, the plant was known by two scientific names considered synonyms, *Malpighia glabra* L. and *Malpighia puniceifolia* L., that were later standardized with the scientific name of *Malpighia emarginata* DC. [109]. It is found from southern Texas (USA), Mexico, and Central America to some subtropical Asia, India and South America, being Brazil the greatest exponent and one of the world largest producers [110-111].

Regarding from nutritional and botanical aspects to industrial-technological (processing), it is observed that the fruit presented a short post-harvest period (2-3 days) at ambient temperature and its maturing process involves a number of chemical reactions, being the primary the conversion of chloroplasts to chromoplasts with concomitant carotenoids, anthocyanins and phenolics production, besides volatile compounds. The whole process of maturing leads to the peculiar characteristics of flavor of ripe fruit [112]. On the market, the most common forms of commercialization are the *in natura* and frozen pulp and juice [113].

Fruit consumption is associated with health benefits, such as reduced risk of cancers, arterial hypertension and other heart disease [111; 114], which come to its high nutritional value and the presence of compounds antioxidants, mainly due to the high content of ascorbic acid (vitamin C), whose levels vary between 300 and 4600 g 100 mg⁻¹ of fruit, being one of the most important natural sources of this vitamin [115-117]. Acerola fruits also offer other important nutrients, such as anthocyanins (phenolic compounds belongs flavonoids class, which combined with the carotenoids, are responsible for fruit colorants), carotenoids, minerals (phosphorus, iron and calcium) and B

vitamins (thiamine, riboflavin, niacin) [115; 117-120]. Carotenoids are present in levels ranging to 371 and 1881 mg 100 g⁻¹, being beta-carotene the majority carotenoid (40-60% of total carotenoids) [121-122]. In relation to the flavonoids, the main class is represented by anthocyanins (37.9–597.4 mg kg⁻¹) and flavonols (70–185 mg kg⁻¹) [123-125].

The high antioxidant content of acerola allows the realization and development of technologies and studies aiming at to obtain and apply these compounds in food matrix as an alternative to synthetic antioxidants commonly used by the industry. This is mainly due to antioxidant action of its components, whose mechanism of action is based on the inhibition of oxidation in cells and biomolecules such as DNA, proteins, lipids, by eliminating free radicals formed, responsible for biological systems oxidation [126-127]. This action of antioxidant compounds is related to health benefits, reported previously, that are prevent hypertension, arteriosclerosis and myocardial infarction [128-130].

3. Oxidative stress and oxidation in biological systems

Oxidative stress is defined how the status of the organism that involves cellular damage, through the release of free radicals or not radical oxygen species without scavenging and/or neutralization factors, configuring a disproportion in redox reactions (oxidation), an imbalance of the oxidizing and reducing factors in biological systems and foods [131-133]. It is considered an interruption of redox circuits that are part of the signalization of the signal transduction pathways, such as cysteine portions regulated by glutathione or tioredoxines. This definition led to the creation of methods to distinguish its redox signaling disruptions and thus control them. It is a response to the simple and viable markers in research and treatment of diseases, whose perpetrators are the reactive oxygen and nitrogenous species [132-133]. These reactive species can be represented by unstable radical containing at least one unpaired electron or by oxidation of radical species, which can promote the lipid peroxidation of membrane with lipid peroxides accumulation [134-136].

During cellular respiration, there is the unpaired electron transfer to molecular oxygen, generating free radicals and reactive oxygen molecules produced during the process [137]. These reactive species are present at physiological levels during the normal operation of cells and can react with some immune system cells (immune receptors) [138]. However, in excessive amounts these species are able to attack vital biological molecules such as nucleic acids, lipids (mainly polyunsaturated fatty acids), proteins, and carbohydrates. It is known that, since the DNA is damaged is susceptible to mutations, and consequently arise physiopathology of several diseases such as: heart disease (atherosclerosis, cardiorespiratory insufficiency), type 2 diabetes, neurodegenerative diseases (Alzheimer, Parkinson), infections/inflammations, and cancer [136; 139].

Different types of oxygen derivatives free radicals, called reactive oxygen species (ROS), include superoxide anion radicals (O₂^{•-}), hydroxyl (OH[•]), peroxy (ROO[•]), alkoxy (RO[•]), and nitric oxide (NO). Among the free radicals, hydroxyls and alkoxy present high reactivity in biomolecules, whereas superoxides, hydroperoxides and nitric oxide present less reactivity [140]. In parallel with the

reactive oxygen species radicals, we also have not reactive oxygen species radicals, such as oxygen singlet, hydrogen peroxide and hypochlorous acid [83].

During normal biologic processes, ROS are formed in small quantities, and antioxidant systems of the organism can neutralize them. However, under conditions of stress such as drugs ingestion, metabolic disorders or UV radiation exposition, these ROS can be generated in quantities that exceed the normal defense capability of the antioxidants, causing the oxidation of biomolecules and initiation of oxidation in the tissues [141-142] In these circumstances, antioxidants present in the diet, slow chain reactions of oxidation, acting in initiation and/or propagation stages of the oxidative process [45; 135; 143).

4. Lipid oxidation and antioxidants use in foods

During storage, the edible oils and fats, as well as lipid-based foods, undergo oxidation and products are formed, causing oxidative rancidity and reduced sensorial properties in foods. Rancidity and off flavor products are produced as well as potentially toxic products [144]. It is known that the autoxidation process (oxidation/redox) is a set of chain reactions of lipids oxidation in the presence of catalytic oxidizers (light, oxygen, temperature, metals), in order to stabilize unstable molecules formed during the chain reactions, such as free radicals. In foods, the main oxidizer is usually the molecular oxygen present in the air, and the main catalysts are light and certain metal ions [145].

The set of chain reactions that occur during lipid oxidation is separated into three phases known as initiation, propagation and termination. The lipid autoxidation starts through a reaction of an unstable radical (oxygen radicalar, for example) with an oil that contain significant quantity of unsaturated fatty acids (double bonds between carbons of the chain) can be mono or polyunsaturated, and the more unsaturated the more unstable and susceptible are the lipid. Therefore, initiation occurs when the fatty acid that composes the lipid has hydrogen removed from the methyl end (CH_3) of your chain for an unstable radical and from this beginning a series of reactions that are triggered in order to stabilize radicals formed. There are several initiators of this process in foods, previously cited, however the main ones are light (UV), transition metal ions, and certain enzymes. The products formed during lipid oxidation are responsible for the loss of food and nutritional quality and gives the rancid and oxidized characteristic to the lipid-based foods [144].

In order to avoid or reduce the reactions of oxidative process, it is necessary to stabilize the food lipids through the use of antioxidants (natural or synthetic), classified into groups according to the mechanism of action (Table 1) [49].

Table 1. Classification and mechanism of action of lipid oxidation inhibitors

Type of inhibitor	Mechanism of action
Antioxidants	Reaction with free radicals, interrupting the propagation phase of the chain reaction
Synergists	Increasing antioxidant activity
Retarders	Reducing hydroperoxides without forming free radicals
Metal scavengers	Inhibiting the ability of heavy metals to catalyze the production of free radicals
Singlet-oxygen quenchers	Deactivating singlet oxygen, which may initiate the free-radical chain reaction

POKORNÝ (1991)

Studies related to antioxidants uses to prevent/reduce the oxidative rancidity in lipid-based foods were initiated 60 years ago. Several natural substances have been studied since then; however, synthetic compounds presented advantages for the food industry. Some of advantages are availability, regular quality, reduced price and high antioxidant potential, many times greater than natural substances. Gradually, the legislation in this area has been adapted to allow the use of animal-tested products with assured safety. As a result, increasingly complex and time-consuming tests have been required to ensure toxicological safety regarding the use of synthetic substances as antioxidants in foods [146].

Since the decade of 70, consumers and regulatory agencies (legislation) began questioning the safety of synthetic substances, even though in the past have been considered safe. There are efforts by the industry to replace the synthetic antioxidant for natural alternatives, since synthetics require expensive tests to prove harmlessness, and your use being contested and not accepted by most of consumers [147].

Antioxidant agents of greater acceptance are common food ingredients, whose use is not restricted or prohibited by law. Many foods contain compounds with antioxidant activity (Table 2), however can promote sensory changes in the final product, changing, for example, taste, aroma and color and for this reason some of them have your use limited, moreover, often presents low antioxidant activity or even low solubility in lipids, resulting in limited use in oils and fats stabilization, although they can be used in lipid basis foods [49].

Table 2. Natural sources of antioxidants

Sources	Oxidation inhibitors
Oils and oilseeds	Tocopherols and tocotrienols, sesamol and related substances, olive oil resin and phospholipids
Oat and rice brans	Various lignin-derived compounds
Fruits and vegetables	Ascorbic acid, hydroxycarboxylic acids, flavonoids and carotenoids
Spices, herbs, tea and cocoa	Phenolic compounds
Proteins and protein hydrolysates	Amino acids, dihydropyridines, Maillard reaction products

POKORNÝ (1991)

"Identical to natural" substances or purified extracts obtained from edible raw materials can be used as natural antioxidants. They also can be prepared from raw materials generally not used as food. An example is the NDGA (Nordihydroguaiaretic acid), a pyrocatechol isolated from a plant of the genus *Larrea*, who was one of the first known natural antioxidants. The use as additive of natural and synthetic products is allowed, however, questions about food safety have resulted in a ban on certain products in certain countries, which once again demonstrates the need for toxicological and ensuring food safety and harmlessness for one compound when choosing an additive for use in food (Figure 2) [49].

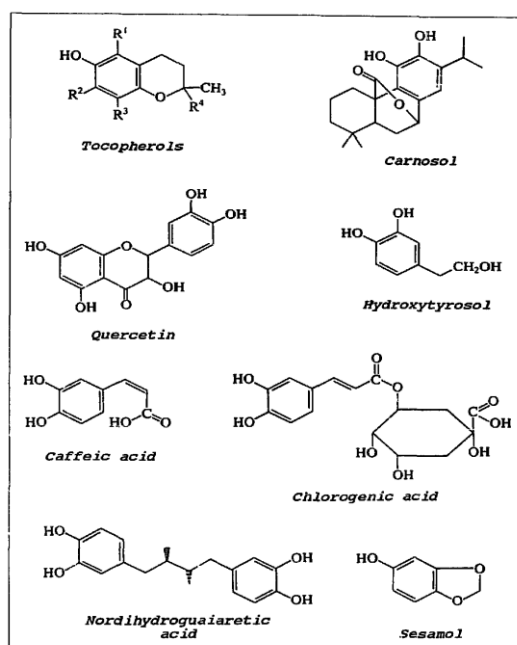


Figure 2. Chemical structure of some natural antioxidants (POKORNÝ, 1991).

An effective way to prevent and reduce lipid oxidation in food includes processes of minimizing loss of natural tocopherols of foods, elimination of the contamination by metals and addition of

antioxidants (natural). Many researches have been conducted to better understand the basic processes of lipid oxidation of polyunsaturated fatty acids, antioxidant action, and effects of lipid oxidation decomposition products during oxidative process. To better understand the action of antioxidants and their effects it is important to get specific information on chemical structure and inhibited oxidation products. Several tests are necessary to elucidate the mechanisms by which oxidation products act on lipid oxidative deterioration of foods [148-149].

The tocopherols are a class of most important natural antioxidants found in food derived from vegetable oils. They have the ability to disrupt lipid autoxidation due to the role in the chain of oxidative reactions, in steps of propagation and decomposition. The α -tocopherol in high concentrations inhibits the hydroperoxides decomposition. Ascorbic acid, another important natural antioxidant, acts synergistically to tocopherols (regenerating them, for example). Ascorbic acid also present multifunctional complex effects and may act as an antioxidant, pro-oxidant, metal chelator (inactive metals initiators), reducing agent (for example, reduce the hydroperoxides) or as oxygen scavenger [149].

5. Lipid emulsions and oxidation

A lipid emulsified system presents three components that determine its behavior: the lipid component (oil phase), the interfacial material or emulsifier (which promotes interaction between lipid and aqueous phase) and the aqueous component. Each of these can have complex chemical composition. Lipid phase, for example, can be partially or fully crystalline and it is subjected to chemical changes such as oxidation or lipolysis; the aqueous phase may contain ions, which are responsible for destabilizing the emulsions. To understand the functional properties of these systems it is necessary to understand the properties of each component individually and together [150].

An emulsion consists of two non-miscible liquids (usually water and oil) dispersed in each other in the form of small spherical droplets. In most foods, the diameter of these droplets ranges from 0.1 to 50 μm . A system that consists of oil droplets in an aqueous phase is known as oil-in-water emulsion (o/w), and some examples are mayonnaise, milk, and cream soups. A system that consists of water droplets dispersed in an oily phase is known as water-in-oil emulsions (w/o) and has as an example the margarine and butter. Is a thermodynamically unstable system due to the free energy needed to increase the surface area between the oil and water phases to become miscible. Over time, emulsions tend to separate in a system that consists of a layer of oil (less dense) above a layer of water (highest density) [144; 151].

To obtain kinetically stable emulsions for a significant period (days, weeks and even months), chemicals known as emulsifiers should be added prior to homogenization. The use of emulsifiers necessary to make miscible the phases one each other. They are molecules that act on the surface of the droplets, are adsorbed on the surface of the droplets formed during homogenization, forming a "membrane" protector that prevents the droplets come near enough to aggregate and separate [151]. The most common used in the food industry are amphiphilic proteins such as casein and whey, soy or

egg phospholipids (lecithin) and small molecules such as surfactants, spans, tweens or fatty acids [144].

Due to the instability of food emulsions, some factors are important in the interaction among particles of fat and on the stability of these systems as the concentration of protein (emulsifiers), temperature, concentration of particles of fat and not adsorbed particles [152]. Koczko and collaborators [153] reported a stability mechanism for emulsions, which involves the micro involvement by micelles of sodium caseinate, in the form of thin films between the particles of fat. The existence of this thin protective film between the fat droplets prevents them approach and has an increase in stability of the emulsion formed.

The oxidative stability of food emulsion is also important for the food industry. The process of oxidation of unsaturated fatty acid chain is different in an emulsified system compared to a bulk vegetable oil. There are techniques to monitor the development of the oxidative process in oils and fats that can be applied in emulsions if lipid fraction is recovered from system before carrying out the analytical determination. These techniques measure the changes of concentration of molecules that are indicative of oxidative process, some of them measure the loss, for example, of reactive starting molecules (oxygen, lipid). Other measure the formation of primary and intermediated oxidation products (conjugated dienes and hydroperoxides) and also secondary oxidation products (alcohols, aldehydes, and ketones hydrocarbons) [144].

6. Accelerated shelf-life tests for lipid oxidation determination

Rancidity in edible oils and lipid-based food occurs due to oxidation reactions and is a common problem in the food industry. There are three main related to it: the presence of polyunsaturated fatty acids (such as omega-3 and omega-6 or *w*-3 and *w*-6 respectively) in food formulation, the decrease or elimination of synthetic antioxidants, and iron fortification of certain foods (such as wheat flour for example). Lipid oxidation not only produces odors and *flavors* of rancid, but also affects the quality and nutritional security through production of secondary oxidation compounds formed after baking or processing [148-149; 154].

Due to the negative effects of lipid oxidation in food, it is essential that the industry make studies on the oxidative stability of the lipids that make up foods, especially those more susceptible to this problem before sells them. For that, the industry seeks more accurate methods of estimating the stability and *shelf-life* that are realized in a relative short period. Some accelerated tests are available to determine *shelf life* and oxidative stability of lipids (ASLT, accelerated *shelf-life* test). It is known that the oxidation reactions increase exponentially with the temperature, and therefore this parameter is typically used to accelerate the oxidative process in most of lipid stability tests [154-156].

Methods of accelerated *shelf-life* or stability have been studied comprehensively by Ragnarsson and Labuza [154] and Rossell [156]. However, the authors recognized that the conclusions reached in many studies of oxidation may not be valid because of the choice of inappropriate methods for evaluation of oxidative stability. Thus, the interpretations of lipid oxidation data must take into account

the limitations of used method [157-159]. Traditional methods of stability are listed in the table below (Table 3) and presented in increasing order of severity of oxidizing conditions used [148-149].

Originally, these methods were developed to be used for homogeneous lipids, such as animal fats and bulk vegetable oils. Unfortunately, these methods present disadvantages, and only a few older studies such the related by Pohle and collaborators [160] and Paul and Roylance [161] were carried out in order to evaluate them critically. Pohle and collaborators (1964) concluded from their results that to in order to reach any satisfactory information of accelerated methods, each of the tests must be adapted to each product to be studied and must take into account mainly the lipid profile of products. Another drawback is that these methods, with the exception of *active oxygen method* (AOM), are not standard methods. Therefore, analysts need to adapt them considering the formulation and product type in study [154].

Although stability tests in normal conditions can get closer to the actual conditions of food storage, the procedure is very slow to be getting a practical value/application. In addition to this negative aspect, the reproducibility of the results is compromised by many variables, which are difficult to control in extended periods of storage. Another factor to consider is that due to the current interest in the use of natural antioxidants in food and the development of new oils and vegetable oil blends, it is appropriate to re-evaluate the current methods for evaluating the oxidative stability of food and edible oils and assess the effectiveness of antioxidants [148].

There are four parameters to control in accelerated *shelf-life* test: temperature, oxygen pressure, addition of metals (pro-oxidants), and contact with accelerated reagents. The reaction rate increases exponentially with increasing temperature and, therefore, the *shelf life* should decrease logarithmically with increasing temperature [148; 154]. Lipid oxidation of single component systems can be represented by the following equation:



Where: RH = polyunsaturated fatty acid (PUFA)

O₂ = oxygen

ROOH = hydroperoxides (primary product)

To estimate the stability or susceptibility to an oxidation, the sample is subjected to an accelerated oxidation test in standardized conditions and an appropriate endpoint must be chosen to determine signs of oxidative deterioration. Therefore, these tests should be appropriate to the formulation, and the conditions used kept as close as possible to the storage conditions of the product. With a view to the practical application of the tests, results and predictions about the oxidative stability in lipid foods and bulk oils should have correlation with the *shelf-life* of the product and the induction period (IP), determined as the time necessary to achieve the oxidation end point. The first step in a typical *shelf-life* is to select an appropriate method to test the food product. A sample is placed under the conditions of time and temperature of the test and the results of induction/incubation time are translated or converted into a *shelf-life* of the product, measured as months of storage, which is

usually done arbitrary and based on the experience of who is performing the analysis or test. As the lipid-based food feature highly susceptible to oxidation of their lipids the use of methods as a way to evaluate the effectiveness of antioxidants in preventing/reducing the reactions of oxidation process is normally applied [148; 154].

Table 3. Conditions and characteristics of stability accelerated tests

Test	Conditions	Characteristics
Ambient storage	Ambient temperature and atmospheric pressure	Too slow
Light	Ambient temperature and atmospheric pressure	Different mechanism
Metal catalysis	Ambient temperature and atmospheric pressure	More decomposition
Weight gain	30-80°C and atmospheric pressure	Questionable end point
Schaal Oven test	60-70°C and atmospheric pressure	Minor or few problems
Oxygen absorption	80-100°C and atmospheric pressure	Different mechanism
Oxygen pump (ASTM*)	99°C and 65-115 psi of O ₂	Different mechanism
Active Oxygen (AOM)	98°C and air bubbling	Different mechanism
Rancimat**	100-140°C	Questionable end point

* American Society for testing materials

** Produced for Metrohm Lid®, Herisau, Switzerland.

FRANKEL (1993,1996)

The methods that use oxidation catalyzed by light or metal provide quick results. However, the photo-oxidation mechanism is different from auto-oxidation of free radicals, which usually occurs in food. The photo-oxidation results in the formation of different flavor with different volatile products formed [162]. Similarly, the oxidation catalyzed by metal can result in a higher proportion of carbonylic products related to the level of primary hydroperoxides [148].

The method of weight gain, based on the increase in weight due to the absorption of oxygen, is not very sensitive. The final point requires a level of oxidation that is beyond the point where the deterioration of taste is detectable in polyunsaturated oils. The schaal oven test conducted at 60-70°C features less limitations associated to hydroperoxides content, because the end point may represent a lower oxidation degree correlated to real storage conditions. At 60°C several secondary reactions are minimized [154].

Stability tests using high temperatures, including the method of oxygen uptake, oxygen pump, *active oxygen method* (AOM) and Rancimat are unreliable, since the mechanism of lipid oxidation change significantly under high temperatures. Volatile acids content is measured automatically by the AOM and Rancimat [163]. These types of tests present limitations, which include [148]:

1. Oxidation rates become dependent on the concentration of oxygen, due to the fact that the solubility of oxygen decrease at high temperatures
2. Oxidation occurs quickly and results in drastic changes in the availability of oxygen

3. The Induction period occurs at a high oxidation level and above the point of detection of rancidity flavours
4. Secondary reactions, polymerization, and cyclisation become important, because in the normal storage temperatures are not relevant
5. The analysis of oxidation under these conditions presents results that are questionable
6. Volatile synthetic antioxidants such as hidroxianisol hydroxytoluene (BHA) and butylated hydroxytoluene (BHT) are subject to significant losses at high temperatures and some phenolic compounds of natural extracts decompose at high temperatures.

Conclusion

The questions raised about the use of synthetic substances as antioxidants by the food industry in relation to safety for human health, showed there are many studies aiming to find natural substitutes, obtained from natural plant sources. Natural sources present a considerable range of bioactive compounds in its composition, whose main representative in acerola is the ascorbic acid (vitamin C) and phenolic compounds (acids phenolics and anthocyanins). This review allowed to conclude and verify the potential of vegetables matrices as antioxidants for the food industry. However, further studies are required due to the diverse and complex chemical composition, which is strongly influenced by several factors, in order offer a feasible alternative to replace synthetic antioxidants in food industry.

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CHAPTER 2. ACTIVE COMPOUNDS EXTRACTION FROM PULP AND SEEDS OF ACEROLA FRUITS AND THEIR PERFORMANCE IN OIL IN WATER EMULSION

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Original paper submitted to “Food Chemistry”

Abstract

Natural sources of antioxidants, including Acerola fruits (*Malpighia emarginata* D.C.) pulp and seeds, present a potential alternative to synthetic products used by food industry due to the high amounts of antioxidant compounds, although the extraction process efficiency and food application are still a challenge. The use of statistical techniques such as experimental design allows the identification of optimal conditions for the recovery of active compounds from new vegetable sources. Optimization study allowed to identify a significant influence of the extraction solvent used and sample to solvent ratio in recovering antioxidants compounds. The optimum extraction condition was by using ethanol and water solution (50% ethanol v/v) and sample: solvent of 1:20 (m/v) at 40°C. Extracts obtained under these conditions were applied to oil in water emulsion, subject to accelerated storage (40 °C up to 9 days). Oxidative stability evaluated by the quantification of hydroperoxides and conjugated dienes during the storage the potential of acerola pulp extracts as antioxidant in sunflower, canola, and corn oil emulsions.

Keywords: Red berries; Antioxidant capacity; Optimization; Lipid oxidation

1. Introduction

Lipid oxidation occurs both in biological systems and in lipid-based foods such as oils and emulsions and consists of a set of oxidation-reduction reactions chain. It is a slow process developed during the three stages, which are induction (catalyzed by light, oxygen and metal ions), propagation, and termination. In foods it is responsible for the formation of undesirable chemical compounds, which are often responsible for off flavors and taste in food, and may be harmful to health (Shahidi, Janitha, & Wanasundara, 1992).

In order to avoid and reduce the oxidation reactions in lipid-based food products, e.g. emulsions, the industry use antioxidants compounds, in most of cases synthetics substances that present preservative function, stabilizing or neutralizing free radicals and consequently increasing the

shelf life in food products. Many methods have been developed to study the oxidative stability to evaluate the evolution of the process in the presence of antioxidant substances in the system. These methods assist in the evaluation of the antioxidant capacity of isolated compounds and plant extracts as measured by oxidation products such as hydroperoxides and other products of lipid deterioration, such as conjugated dienes (Brand-Williams, Cuvelier, & Berset, 1995). Regarding the methods of evaluation of the antioxidant potential of biological samples, they are based on two main mechanisms of action: transfer or donation of hydrogen atom (HAT) and electron transfer (ET) (Huang, Ou & Prior, 2005). Most of the HAT tests present as a mechanism the competition between antioxidant and substrate by the free radical formed, which may be in the case of the ORAC test the peroxy radical, which is formed by the thermal decomposition reaction of an azo compound. The ET assays are based on colorimetric reactions and evaluate the ability of an antioxidant compound to reduce an oxidant, which when reduced modifies the coloration, promoting a discoloration, and with that correlates the concentration of antioxidants with the different grades change of color. Antioxidant capacity measurement methods equivalent to Trolox (TEAC), such as DPPH, ABTS and FRAP assay are the main known ET assays, as well as antioxidant capacity assessment methods by oxygen radicals, assays typically of the HAT type, ORAC is best known and used (Zulueta, Esteve, & Frigula, 2009).

Fruits and vegetables are highly perishable, and they are often submitted to different processing technologies in order to preserve the quality and extend the shelf life. One of the most used is dehydration and/or drying (Zotareli, Porciuncula, & Laurindo, 2002; Lüle & Koyuncu, 2015). However, drying methods that use high temperatures can cause decay and loss of thermo-sensitive compounds including those that present antioxidant action, known to reduce and/or eliminate the oxidative processes in biological systems and in foods, which are started by free radicals and other reactive species (Fujita et al., 2013). The expansion of the industrial use of acerola, fruit from tropical America with extensive cultivation in Brazil presenting high ascorbic acid content, is related to their process to juice, jelly and jam. There is high amount of residues generated after processing with are normally discarded, causing losses of raw materials and energy and consequently generate environmental impacts, which may represent up to 70% of the total volume produced (Da Silva et al., 2013; Cunha et al., 2016). Studies about fruit residues showed high antioxidant potential in acerola waste, associated to the presence of phenolics, flavonoids and ascorbic acid (Barrozo, Santos, & Cunha, 2013; Bortolotti et al., 2013; Barrozo et al., 1996; Duzzioni et al., 2013). Another component of the waste of acerola is water, which can represent up to 80% of the composition, a fact that limits the *shelf life*, transport and storage of these residues (Duzzioni et al., 2013). Accordingly, there is a necessity to use drying methods, such as *spray dryer* (atomization or micro-encapsulation) or *freeze dryer* (lyophilization) for a better industrial application.

This set of factors demonstrates the necessity of statistical tools used in experimental planning (factorial or univariated) and optimization of experimental parameters, which are essential for the proper understanding and analysis the results and information generated (Cunico et al., 2008; Pereira-Filho, Poppi, & Arruda, 2002). This tools are considered critical in the development of research focused on new technological processes with a view to large-scale application (Pereira-Filho, Poppi, & Arruda,

2002; Peralta-Zamora, Morais, & Nagata, 2005). Within the experimental statistics, factorial planning is of greater relevance in scientific research, as it allows to study at the same time a large number of variables, from a reduced amount of experimental trials, when compared to processes that evaluate only a single variable (Cunico et al., 2008; Pereira-Filho, Poppi, & Arruda, 2002; Peralta-Zamora, Morais, & Nagata, 2005).

The main objective of this study was to apply optimization techniques on extraction process of natural antioxidants from pulp and seeds of acerola fruits, studying the variables temperature, ethanol concentration and sample: solvent ratio and as response the reducing power and antioxidant activity by DPPH and ABTS. In addition, a chemical characterization was done by. Finally, the study of oxidative stability used schaal oven test, evaluating two oxidation products (hydroperoxides and conjugated dienes) in a model lipid system added off natural extracts obtained from acerola (pulp and fruit) and synthetic antioxidants (BHA and BHT).

2. Material and methods

2.1 Sample preparation and characterization

The acerola fruits and commercial pulps were provided by the agricultural cooperative of Junqueirópolis (COOPAJ), located in the city of Junqueirópolis/SP. The fruits were kept under refrigeration (5°C) until the washing and sanitization in chlorinated water (200 ppm of sodium hypochlorite) by 10 minutes and then stored in laminate polyethylene packages until the physicochemical characterization.

To realize physicochemical characterization of pulp and fruit from acerola analyses of color, pH, content of total soluble solids (TSS), titratable acidity (TA) and ratio (TSS/AT) were performed. The color was measured in colorimeter (Konica Minolta 200b) and the color parameters evaluated were L^* , a^* , b^* Hue and Chroma, where L^* represents lightness or brightness, Hue is the tonality angle (tone), Chroma indicates the chromaticity or purity/saturation of the color. The lightness (L^*) ranges from 0 (black) to 100 (white) and the parameters a^* and b^* , which are related chromaticity or color, vary from red (+) to green (-) and from yellow (+) to blue (-) respectively (Minolta, 1998). Six measurements were made of the color parameters for each sample. The pH measurement was performed using pH meter (Tecnal Tec-3MP) in diluted 10 times samples (10 grams: 100 mL of distilled water) according to the AOAC (2005), and in triplicate. The content of soluble solids total (SST) was determined using a portable digital refractometer (0 to 99 ° BRIX) (Kruss DR201-95), and the results expressed in °BRIX according to AOAC (2005). For titratable acidity analysis (TA) a NaOH 0.1M solution was used to neutralize pH up to 8.1-8.2 in samples diluted ten-fold (10: 100), following the AOAC (2005) and the results expressed as grams of citric acid per a hundred grams of sample ($\text{g citric acid } 100 \text{ g}^{-1} \text{ sample}$). The ratio (SST/TA) was calculated from the ratio of the total soluble solids content with the titratable acidity.

In order to obtain the pulp, the fruits were manually stripped, then the pulp were lyophilized and ground in a stainless steel blender and packed in laminated polyethylene

packages. The commercial pulp was kept frozen (-22°C), then lyophilized and stored in laminated polyethylene packages. The lyophilized fruits, pulps, and seeds were stored in a freezer at -22°C until the analyses. To obtain the extracts, samples have been added of the solvent (aqueous ethanolic solution) in the proportion defined in experimental design (m/v), in bath dubnoff with shaking of 200 rpm, and temperatures also defined by experimental design. Subsequently, the extracts were centrifuged at 5000 g for 20 minutes at 4°C (Eppendorf), filtered and stored in amber vials at -22°C until the realization the analyses. The responses studied in the design were reducing power (total phenolic compounds content) and antioxidant capacity by DPPH and ABTS.

2.2 Experimental design

A central composite rotatable design (CCDR) was applied, involving three independent variables (2^3) and five levels for each exploratory variable. The study ranges for variable temperature was 30°C to 60°C . The degree of ethanol hydration (ethanol concentration) ranged from 0% to 95% and sample: solvent ratio (in mass/volume or m/v) from 1:10 to 1:30.

In total 18 runs were carried out, including four repetitions of the central point (Table 1). All experiments were conducted in random way and using the same equipments. The effects were analyzed by response surface methodology and multiple regression analysis in Statistica software (Statsoft, 2001). The mathematical models were adjusted, including linear, quadratic terms and interactions of exploratory variables, and in relation to the coefficient of determination (R^2) and analysis of variance (ANOVA and F-test).

The responses were the reducing power and *in vitro* antioxidant activity (DPPH and ABTS). The reducing power in the extracts was performed by the method of Singleton, Orthofer, and Lamuela-Raventos (1999), which using the method of Folin-Ciocalteu, with gallic acid as standard and reading in spectrophotometer at 765 nm. The results were expressed in mg of gallic acid equivalent (GAE) per gram of sample (mg GAE g^{-1}). Antioxidant activity was evaluated by DPPH and ABTS methods, with Trolox as standard (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The method of DPPH was conducted according to methodology of Brand-Williams, Cuvelier, and Berset (1995) adapted by Kim et al. (2002). The method is based on the ability of antioxidant in reducing the oxidized radical DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) by means of hydrogen donation. This reaction is evaluated by radical discoloration and absorbance reading in spectrophotometer at a wavelength of 515 nm after 45 minutes of the reaction.

The ABTS method used was described by Re et al. (1999) modified by Kuskoski et al. (2004). The method presents the same principle of DPPH, having the same mechanism of hydrogen donation to the reduced radical ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)), promoting its discoloration that is quantified in spectrophotometer with absorbance in 734 nm 6 minutes after the reaction. The results for the DPPH and ABTS methodologies were expressed as Trolox equivalent (TEAC), in $\mu\text{mol Trolox g}^{-1}$ sample.

Table 1. Experimental design of antioxidants extraction in pulp and seed of acerola.

Run	Independent Variables					
	Ethanol Concentration (%)		Temperature (°C)		Sample: solvent (m/v)	
	Coded Value	Real Value	Coded Value	Real Value	Coded Value	Real Value
1	-1	19	-1	36	-1	1:14
2	1	76	-1	36	-1	1:14
3	-1	19	1	54	-1	1:14
4	1	76	1	54	-1	1:14
5	-1	19	-1	36	1	1:25
6	1	76	-1	36	1	1:25
7	-1	19	1	54	1	1:25
8	1	76	1	54	1	1:25
9	-1.68	0	0	45	0	1:20
10	1.68	95	0	45	0	1:20
11	0	47	-1.68	30	0	1:20
12	0	47	1.68	60	0	1:20
13	0	47	0	45	-1.68	1:10
14	0	47	0	45	1.68	1:30
15	0	47	0	45	0	1:20
16	0	47	0	45	0	1:20
17	0	47	0	45	0	1:20
18	0	47	0	45	0	1:20

2.3 Extract preparation and characterization

After the surface responses analysis, the ideal condition was selected and the mathematical model was validated by the determination of the reducing power, expressed in milligrams of gallic acid equivalent per ml of extract (mg GAE ml⁻¹) and antioxidant activity by DPPH and ABTS.

Additionally, the following determinations were performed: Antioxidant activity by ORAC method, quantification of phenolic and ascorbic acid, and anthocyanins quantification.

The antioxidant activity by FL-ORAC (oxygen radical absorbance capacity) in hydrophilic and lipophilic medium (H-ORAC and L-ORAC respectively) and FRAP (ferric reducing antioxidant power) were held in reader spectrophotometer microplate (Synergy HT, Biotek, Winooski, USA) with integrated data analysis software Gen5™ 2.0. The results were expressed as μmol of Trolox equivalents per ml of extract, using Trolox standard curve for each assay (TEAC Trolox $\mu\text{mol ml}^{-1}$). For FL-ORAC, the fluorescein was used as a fluorescent molecule indicator and Trolox solution as standard. The area of the curve was used for the calculations and described by Dávalos, Gómez-Cordovés, and Bartolomé (2004). The wavelengths used were of 485 nm to excitation and 520 nm to emission, being the decay of fluorescence read every 1 minute during 80 minutes.

The method used for H-ORAC was proposed by Ou, Hampsch-Woodill, and Prior (2001) and Ou et al. (2013). It was added 20 μL of extract or standard solutions, in this case Trolox, 125 μL of fluorescein diluted in phosphate buffer (pH 7.4) and 25 μL of the radical source AAPH (2,2'-azobis(2-methylpropionamidine) dihydrochloride) in black background microplate. For L-ORAC Ou and others (2001) protocol was adapted by Huang et al. (2002) and Ou et al. (2013). The apolar extract, more lipid-soluble (hexanic) was diluted in RMCD 7% solution (Randomly metilated β -cyclodextrin) (RMCD

Trappsol®) prepared with acetone solution 50% (v/v). Diluted extracts in RMCD were pipetted in black background microplate along with fluorescein and AAPH. The microplate was read under the same conditions of H-ORAC. The T-ORAC (total ORAC) or FL-ORAC was determined by calculating the sum of H-ORAC and L-ORAC values. The results were expressed in $\mu\text{mol Trolox mL}^{-1}$ of extract.

The FRAP method was performed according to Benzie and Strain (1996) and Rufino et al. (2006; 2010). The FRAP reagent was prepared by mixing 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ solution (2,4,6-tri-2-pyridyl-1,3,5-triazine) in 40 mM HCl and 20 mM iron (III) chloride (FeCl_3) in ratio 10:1:1 respectively. The samples and standard solutions (Trolox) have been added to water and reagent FRAP in microplate and then they were incubated in an oven at 37°C for 30 minutes and later read at 595 nm wavelength. The results of both methods were expressed in Trolox equivalent antioxidant capacity per ml of extract (TEAC) in $\mu\text{mol Trolox mL}^{-1}$ of extract

The quantification of phenolic acids (chlorogenic, ferulic and p-coumaric) and ascorbic acid was performed by high performance liquid chromatography (HPLC) with diode arrangement detector (UV/VIS). The methodology used was described by Lee and Coates (1999). The mobile phase used was trisodium citrate solution 0.01 M (phase A) and methanol (phase B) with the following gradient during 20 minutes of running and 10 minutes of cleaning, totaling 30 minutes: 100% of citrate (up to 1.5 minutes), 30% of citrate and 70% methanol (up to 13 minutes) and 10% of citrate and 90% methanol (from 13.1 minutes), with flow of 1 mL min^{-1} and injection volume of 10 μL . The extract was filtered at 0.45 μm membrane (0.45 μm PTFE, Millipore, Massachusetts, USA) for injection and quantification accomplished through standard curve for each analytical compound (chlorogenic, p-coumaric, ferulic and ascorbic acids). Chromatography analysis followed the same conditions for both phenolic and ascorbic acid.

The determination of total anthocyanins was carried out by the method of differential pH (pH 1.0 and 4.5) described by Wrolstad (1976), with modifications. The solution pH 1.0 was prepared from potassium iodide solution (KCl 0.2 N) acidified with HCl. The buffer with pH 4.5 was prepared from sodium acetate solution (1N), acidified with HCl. Using 1 ml of extract and 4 ml of pH buffer solutions, reading at 520 and 700 nm in a spectrophotometer, the result was expressed in milligrams of cyanidin-3-glucosideo per ml of extract ($\text{mg cyanidin-3-glucosideo mL}^{-1}$). The separation, identification and quantification of the anthocyanins in the pulp and fruit extracts were performed by Ultrahigh performance liquid chromatography (UHPLC) according to Natividade et al. (2013), with modifications. Samples of lyophilized extracts were weighed (0.2 g) and extracted with methanol: water (80:20 v/v) and after filtered through membrane (PTFE, 0.45 μm , Millipore, Massachusetts, USA) and injected (20 μL) in a ultra-high efficiency chromatograph (Ultimate 3000 BioRS, Dionex-Thermo Fisher Scientific Inc., USA), equipped with diodes arrangement detector (DAD) and Luna® column 2.5 μm C18 (2) HST 2.0 \times 50 mm (Phenomenex®, USA) maintained at a temperature of 39°C. Elution of the substance (standards) was made by gradient with flow of 0.6 mL min^{-1} , with readings at wavelengths of 280, 320, 360 and 520 nm. The mobile phase consisted of a phosphoric acid solution 0.85% (solvent A or phase A) and 100% acetonitrile (solvent B or phase B). The gradient used for separation was 0–2.5 min: 4% B; 2.5–7.5 min: 8% B; 7.5–15 min: 12% B; 15–18 min: 15% B; 18–20 min: 20% B; 20–21 min: 25% B; 21–22 min: 35% B; 22–24 min: 65% B; 24–25 min: 65% B; 25–25.5 min: 35% B; 25.5–27 min: 0% B.

The identification and quantification (in triplicate) of substances was carried out by comparing their retention times and the spectrum absorption in ultraviolet region.

2.4 Emulsion preparation

The model lipid systems were prepared in accordance with Huang et al. (1996). Three different types of oils (sunflower, canola and corn) were used, resulting in eight treatments: control without addition of antioxidant, with addition of 200 and 400 mg kg⁻¹ extract of lyophilized acerola pulp, with addition of 200 and 400 mg kg⁻¹ extract of lyophilized acerola fruit, with addition of 100 mg kg⁻¹ (ppm) of synthetic antioxidant BHA, with addition of 100 mg kg⁻¹ (ppm) of synthetic antioxidant BHT and finally with addition of 200 mg kg⁻¹ (ppm) of the mixture of antioxidants synthetics BHA and BHT, being 100 mg kg⁻¹ (ppm) of each, to evaluate the oxidative stability.

The basic formulation of the emulsions followed the ratio of ingredients: 200 g of oil without the addition of synthetic or natural antioxidants (provided by Cargill, Mairinque, SP), 180 g of distilled water and 20 g Tween 20. The mixture was homogenized in Homogenizer Turrax type for 2 minutes to 18000 rpm. The extract, obtained from the pulp of acerola and acerola (whole ripe fruit) lyophilized, were rotaevaporated in rotaevaporator BUCHI (model) before incorporating for the emulsion for withdrawal of all the ethanol present so that it does not interfere in emulsion stability.

2.5 Oxidative stability

To evaluated the efficiency and feasibility of applying natural antioxidant extracts obtained from acerola compared to synthetic antioxidants, oxidative stability studies were conducted by the means of lipid oxidation products analyses (hydroperoxide content and absorptivity at UV) in model emulsified lipid systems (without addition of antioxidants (control) and added of synthetics (BHA and BHT) and natural (derived from acerola products – pulp and whole fruit) antioxidants. The emulsions were stored in oven at 40°C and samples of each treatment collected and frozen at -20°C every three days during nine days of storage. The hydroperoxide content was determined according to Shanta and Decker (1994). To prepare and dilute sample was added 0.3 mL of separated oil emulsion with 1.5 mL of a mixture of isooctane/iso-propanol (3:1, v/v), followed by unrest in vortex (brand/model) (3 times for 10 seconds). For indicator preparation was mixed same quantities of ammonium thiocyanate solution (NH₄SCN 3.97 M) with filtered iron saline solution, this last has been prepared blending same proportions of barium chloride dihydrate solution (BaCl₂·2H₂O 0.132 M) with heptahydrated iron (II) sulfate solution (FeSO₄·7H₂O 0.144 M). An aliquot of the prepared sample (200 µl) was mixed with 2.8 mL of methanol/butane (2:1, v/v), followed by the addition of 30 µl of the indicator, then stirred in vortex and expected 20 minutes for reading in spectrometer with absorbance of 510 nm. The hydroperoxides concentration in the sample was determined using cumene hydroperoxide standard curve. The results were expressed in mmol of cumene hydroperoxide per liter of oil (mmol CHP L⁻¹). The absorptivity in ultraviolet range or conjugated dienes was determined according to method Ch 5-91 (AOCS, 2003). The samples were weighed (0.03 to 0.05 g), diluted in isooctane (2,2,4-trimethylpentane) and the volume was completed to 25 ml in volumetric flask or until

the absorbance read of the dilutions obeyed the Lambert-Beer law (absorbance between 0.2 and 0.8). The absorbance reading was held in spectrophotometer (model) to wavelength of 232 nm using isooctane as blank. The results calculated by means of the following equation:

$$E = \text{Abs}/C \quad \text{Equation (2)}$$

Where:

E = % Conjugated Dienes

Abs = Absorbance in a specific wavelength, 232 nm

C = Concentration of sample solution in g 100 ml⁻¹

2.6 Statistical Analysis

The physicochemical parameters and extract characterization data were analysed by means of a completely randomized type design, evaluated through the SAS software (Sas Institute, 2011) and submitted to analysis of variance (ANOVA and F-test). A Tukey test, at the level of significance of 5% ($p < 0.05$) was performed to determine statistically significant differences between the samples. The analyses were realized in triplicate and was calculated mean and standard deviation for each sample or treatment.

For oxidative stability analyses an experimental design with randomized blocks was used to analyze the results, with three oils, eight treatments for oil and four storage times, all in triplicate. The data obtained, means and standard deviation for the three repetitions, were submitted to analysis of variance (ANOVA and F-Test), and the means compared by the Tukey test with interval of confidence of 5% ($\alpha = 0.05$).

3. Results and discussion

3.1 Experimental design

Factorial planning assists in determining factors that have relevant effects on the desired responses, and how the effect of one factor varies with the levels of the other factors. In addition, it allows establishing and quantifying the correlations between the different factors. Without the use of factorial planning experiments, important interactions between factors cannot be detected and maximum optimization of the system may take longer to achieve. In addition, the observed advantages, factorial planning makes the determination feasible, or at least indicates the optimal condition (Cunico et al., 2008). Results are present in following (Tables 2-5)

Table 2. Reducing power (mg GAE g⁻¹), DPPH and ABTS (μmol Trolox g⁻¹) of acerola pulp extracts

Run	Ethanol Concentration (%)	Temperature (°C)	Sample: solvent (m/v)	Reducing Power (mg GAE g ⁻¹)	DPPH (μmol Trolox g ⁻¹)	ABTS (μmol Trolox g ⁻¹)
	Real Value					
1	19	36	1:14	108.42±3.82	221.82±4.77	417.25±3.30
2	76	36	1:14	103.94±0.17	223.77±0.92	452.16±3.96
3	19	54	1:14	116.04±0.74	221.83±0.82	461.81±0.66
4	76	54	1:14	119.60±4.04	222.90±2.57	421.48±3.94
5	19	36	1:25	106.70±1.77	386.51±0.35	522.05±2.35
6	76	36	1:25	145.03±3.04	404.41±3.90	618.90±4.71
7	19	54	1:25	88.75±1.82	386.38±1.87	423.80±3.53
8	76	54	1:25	138.09±4.66	394.94±1.06	430.95±9.38
9	0	45	1:20	91.72±1.13	313.59±0.56	544.86±4.68
10	95	45	1:20	62.06±3.71	315.00±6.18	584.09±3.75
11	47	30	1:20	104.45±3.09	315.75±1.63	567.56±1.88
12	47	60	1:20	133.35±0.71	309.43±4.49	555.04±3.77
13	47	45	1:10	107.76±0.36	159.86±0.33	284.03±3.77
14	47	45	1:30	132.50±4.02	462.95±5.79	739.18±1.41
15	47	45	1:20	162.80±0.24	312.16±0.57	706.77±3.77
16	47	45	1:20	163.59±2.53	315.31±5.67	707.15±4.71
17	47	45	1:20	169.42±3.83	314.37±1.13	710.35±3.75
18	47	45	1:20	165.27±2.95	316.19±3.16	712.57±3.77

It was observed that for the reducing power that only the quadratic effects of the independent variables (ethanol concentration, temperature, and sample: solvent ratio) were significant, and both presented negative coefficients ($p \leq 0.05$). Ethanol concentration presented the higher effect on the reducing power of the acerola pulp extracts. For the DPPH, only the linear effect of the sample: solvent ratio variable was significant, presenting a positive coefficient ($p \leq 0.05$). For the ABTS variable, the quadratic effects of the studied variables were significant and presented negative coefficients ($p \leq 0.05$). The linear effect of the sample: solvent ratio was also significant, with a positive coefficient ($p \leq 0.05$). For both the ABTS and the DPPH, the sample: solvent ratio was the independent variable with the highest effect for the acerola pulp extracts. The interaction between the factors studied was not significant for any of the response variables.

The analysis of variance (ANOVA) and the F-test were performed, and it was observed that the calculated F for all variables studied (reducing power, DPPH and ABTS) was higher than the F-table, indicating that the regression was statistically significant for both variables, that is, with 95%

confidence ($\alpha = 5\%$), the data are explained by the proposed model. The regression coefficients R^2 of the models, which explain the variation of the data, were 85.45% for reducing power, 99.80% for the variable DPPH and 81.77% for the ABTS variable (Table 3). The mathematical models for the three response variables are presented below and are complete second-order models, in which none of terms was excluded, so that the R^2 value was not decreased and some effect was not erroneously ignored.

Table 3. Variance analysis (ANOVA) of reducing power, DPPH and ABTS variables of acerola pulp extracts

Cause or Source of Variation	Degrees of Freedom	Sum of Squares	Middle Square	Fcalc.	P
Reducing Power ($R^2 = 85.45\%$)					
$Y_1 = 164.59 + 2.70 x_1 - 28.18 x_1^2 + 5.29 x_2 - 12.90 x_2^2 + 3.44 x_3 - 13.33 x_3^2 + 11.07 x_1 x_2 + 2.38 x_1 x_3 - 6.02 x_2 x_3$					
Regression	9	13180.1	1464.5	5.2	0.01467*
Residue	8	2244.9	280.6	-	-
Total	17	15425.0	-	-	-
DPPH ($R^2 = 99.80\%$)					
$Y_2 = 314.74 + 2.33 x_1 - 1.12 x_1^2 + 87.29 x_2 - 2.14 x_2^2 - 1.54 x_3 - 1.73 x_3^2 + 2.93 x_1 x_2 - 1.28 x_1 x_3 - 1.09 x_2 x_3$					
Regression	9	104260.7	11584.5	446.4	0.000000*
Residue	8	207.6	26.0	-	-
Total	17	104468.3	-	-	-
ABTS ($R^2 = 81.77\%$)					
$Y_3 = 712.47 + 12.05 x_1 - 65.75 x_1^2 + 73.84 x_2 - 84.44 x_2^2 - 21.48 x_3 - 66.87 x_3^2 + 13.68 x_1 x_2 - 20.62 x_1 x_3 - 37.51 x_2 x_3$					
Regression	9	242440.8	26937.9	4.0	0.03216*
Residue	8	54053.7	6756.7	-	-
Total	17	296494.5	-	-	-

* Significant for $\alpha = 5\%$ (0.05), $p \leq 0.05$

$F_{0.95,9,8} = 3.23$, $F_{0.95,5,8} = 4.82$

Where: Y_1 is a total reducing power (mg GAE g⁻¹)

Y_2 is a DPPH antioxidant activity ($\mu\text{mol Trolox g}^{-1}$)

Y_3 is an ABTS antioxidant activity ($\mu\text{mol Trolox g}^{-1}$)

x_1 is an ethanol concentration in %

x_2 is a proportion sample: solvent in m/v

x_3 is a temperature in °C

The response surfaces generated for the reducing power, DPPH and ABTS parameters confirm the statistical results, obtained by ANOVA, F test and effects analysis, and allow to conclude that for the reducing power any temperature and sample: solvent ratio can be used from that the ethanol concentration is between 25-75%. There was no optimal range for antioxidant activity by the DPPH method. Any concentration of ethanol and temperature can be used if the highest sample: solvent

ratios are used (1:20-1:30). For the ABTS variable, the sample: solvent ratio was again the most significant exploratory variable (Figure 1).

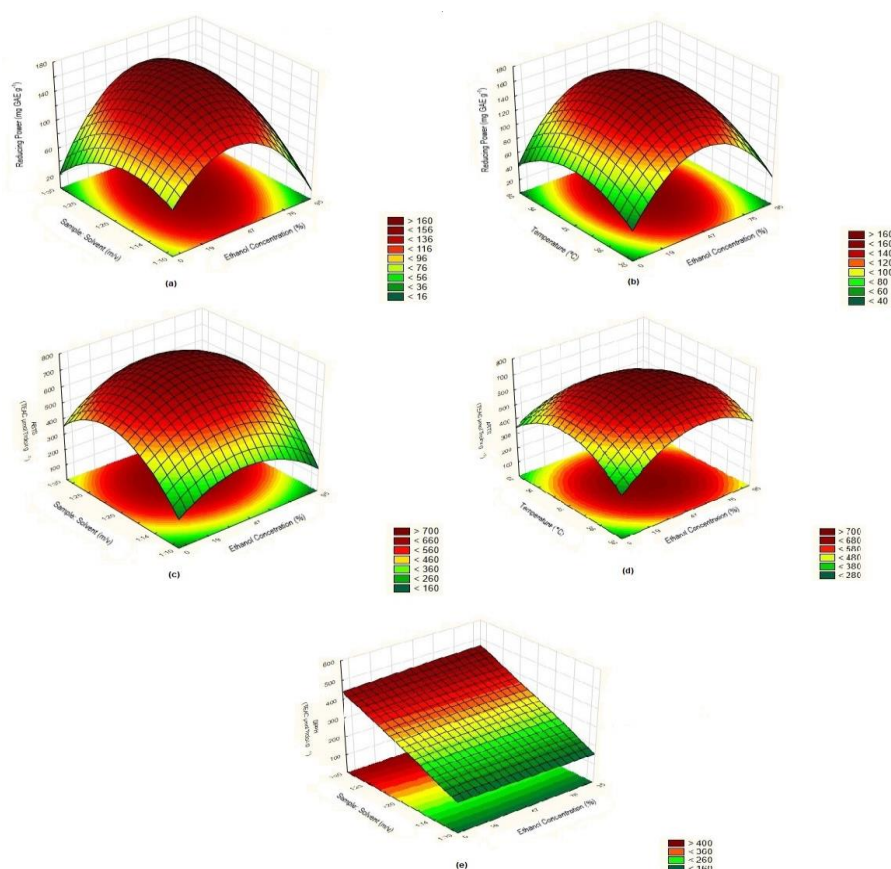


Figure 1. Surface response of acerola pulp optimization for reducing power (a, b), ABTS (c, d) and DPPH (e).

(a) Ethanol concentration x Sample: solvent for reducing power; (b) Ethanol concentration x Temperature for reducing power; (c) Ethanol concentration x Sample: solvent for ABTS; (d) Ethanol concentration x Temperature for ABTS; (e) Ethanol concentration x Sample: solvent for DPPH.

Rezende, Nogueira, and Narain (2017) carried out a study of optimization of antioxidant extraction in acerola pulp processing residues and found greater efficiency and better results when using ultrasonic assisted extraction with the following conditions: ethanol concentration of 46.49%, sample: solvent ratio of 8.66 ml g⁻¹ and extraction time of 49.30 minutes.

3.1.2 Acerola seed

For acerola seeds, lower results of reducing power were observed compared to acerola pulp. In addition, the seeds extracts did not demonstrate lower antioxidant activity. Rezende, Nogueira, and Narain (2017) found the optimum antioxidant extraction conditions in acerola pulp processing residues (skin and seeds) total phenolic contents of 1034 mg EAG 100 g⁻¹ and antioxidant capacity by the

DPPH and ABTS methods of 155 $\mu\text{mol Trolox g}^{-1}$ e 179.8 $\mu\text{mol Trolox g}^{-1}$ respectively, and also observed that the linear effect of the solvent: sample ratio was the most significant variable and influenced the phenolic extraction as well as on the antioxidant capacity (DPPH and ABTS).

For the response reducing power, points in the intermediate condition of ethanol concentration, temperature and sample ratio: solvent (47%, 45 °C and 1:20, central point) and points of 76% ethanol concentration, sample: solvent ratio of 1:25 and temperatures between 36-54°C were those that resulted in higher antioxidant potential of extracts. Points of lower concentrations of ethanol (0 and 19%) and sample: solvent ratio of 1:14 provided lower reducing power in the seed extract (Table 4). Silva, Duarte, and Barrozo (2016) obtained in residues of acerola juice and pulp processing average phenolic content around 726.6-913.6 mg EAG 100 g^{-1} , already the residues of acerola juice processing analysed by Oliveira et al. (2009) presented total phenolic content of 681 mg EAG 100 g^{-1} . For the DPPH response, the intermediate condition of ethanol and temperature and intermediate to highest sample: solvent ratio (47%, 45°C and 1:20 – 1:30) resulted in higher activity. (Table 4).

Table 4. Reducing power (mg GAE g^{-1}), DPPH and ABTS ($\mu\text{mol Trolox g}^{-1}$) of acerola seed extracts

Assay	Exploratory Variables			Response Variables		
	Ethanol Concentration (%)	Temperature (°C)	Sample: solvent (m/v)	Reducing Power (mg GAE g^{-1})	DPPH ($\mu\text{mol Trolox g}^{-1}$)	ABTS ($\mu\text{mol Trolox g}^{-1}$)
	Real Value					
1	19	36	1:14	15.86±0.33	17.18±2.02	18.64±2.42
2	76	36	1:14	25.38±0.83	12.60±0.80	27.39±0.47
3	19	54	1:14	18.84±0.26	11.82±4.17	25.35±2.01
4	76	54	1:14	25.50±0.88	20.74±1.37	22.97±1.18
5	19	36	1:25	19.07±0.41	12.48±2.40	34.83±4.87
6	76	36	1:25	33.87±0.72	22.26±1.42	41.31±2.34
7	19	54	1:25	23.25±0.56	17.66±0.85	21.42±4.97
8	76	54	1:25	31.72±0.98	30.44±0.36	32.63±1.00
9	0	45	1:20	18.16±0.20	14.57±1.75	16.33±1.11
10	95	45	1:20	22.13±0.68	15.61±1.30	22.32±3.19
11	47	30	1:20	25.77±0.78	29.60±1.02	24.10±2.86
12	47	60	1:20	26.89±1.15	29.37±3.35	30.52±1.93
13	47	45	1:10	20.45±0.57	15.97±0.18	16.26±0.85
14	47	45	1:30	31.64±0.98	44.78±3.49	31.21±1.54
15	47	45	1:20	34.09±0.33	29.29±2.94	38.91±0.41
16	47	45	1:20	29.97±0.41	30.25±1.27	35.86±0.78
17	47	45	1:20	28.66±1.47	31.17±0.23	32.65±0.74
18	47	45	1:20	29.39±0.25	29.77±0.67	40.63±1.66

Triplicate mean ± standard deviation (SD)

The analysis of variance (ANOVA) and the F test for reducing power and ABTS, showed that the F calculated from the data was larger than the table F, indicating that the regression was statistically significant, with 95% confidence ($\alpha = 5\%$), that is, with a confidence interval of 95%, the data obtained were explained by the model. The regression coefficient R^2 of the model for reducing

power was 87.50% and for ABTS it was 79.05%. For the variable DPPH, it was observed that the calculated F was lower than the table F, indicating that the regression was not statistically significant, and therefore, the model is not predictive, that is, with 95% confidence ($\alpha = 5\%$), the data are not explained by the proposed model. The regression coefficient R^2 of the model, which explains the variation of the data, was 77.50%. Considering this observation, it was opting to exclude the DPPH variable for optimization (Table 5).

When analyzing the effects, in conjunction with ANOVA, it was observed that only the linear effects of the ethanol concentration and sample: solvent variables were significant for the reducing power and presented positive and negative coefficients ($p \leq 0.05$), respectively. The quadratic effect of the ethanol concentration was also significant, with a positive coefficient ($p \leq 0.05$). For the ABTS variable, the quadratic effects of the ethanol concentration and sample: solvent ratio were significant and showed negative coefficients ($p \leq 0.05$). The linear effect of the sample: solvent ratio was also significant, with a positive coefficient ($p \leq 0.05$). For the seed as well as the pulp, it was verified that the interaction between the variables was not significant for both reducing power, DPPH and ABTS. The ethanol concentration was the variable with the highest effect. The mathematical models presented below for the reducing power, DPPH and ABTS of acerola seed extracts, as well as for acerola pulp, are complete second order models, in which none of terms were excluded.

Table 5. Variance analysis (ANOVA) of reducing power, DPPH and ABTS variables of acerola seed extracts

Cause or Source of Variation	Degrees of Freedom	Sum of Squares	Middle Square	Fcalc.	P
Reducing Power ($R^2 = 87.50\%$)					
$Y_1 = 30.51 + 3.38 x_1 - 3.58 x_1^2 + 3.01 x_2 - 1.50 x_2^2 + 0.51 x_3 - 1.40 x_3^2 + 0.89 x_1 x_2 - 1.15 x_1 x_3 - 0.13 x_2 x_3$					
Regression	9	473.9	52.7	6.2	0.00850*
Residue	8	67.7	8.5	-	-
Total	17	541.6	-	-	-
DPPH ($R^2 = 77.50\%$)					
$Y_2 = 30.44 + 2.10 x_1 - 6.73 x_1^2 + 5.05 x_2 - 1.32 x_2^2 + 1.15 x_3 - 1.64 x_3^2 + 2.28 x_1 x_2 + 2.06 x_1 x_3 + 1.32 x_2 x_3$					
Regression	9	1089.2	121.0	3.1	0.06492
Residue	8	316.2	39.5	-	-
Total	17	1405.4	-	-	-
ABTS ($R^2 = 79.05\%$)					
$Y_3 = 36.75 + 2.50 x_1 - 5.08 x_1^2 + 4.47 x_2 - 3.52 x_2^2 - 0.66 x_3 - 2.25 x_3^2 + 1.42 x_1 x_2 - 0.80 x_1 x_3 - 3.05 x_2 x_3$					
Regression	9	872.8	97.0	3.4	0.05137
Residue	8	231.3	28.9	-	-
Total	17	1104.1	-	-	-

* Significant for $\alpha = 5\%$ (0.05), $p \leq 0.05$

$F_{0.95,9,8} = 3.23$, $F_{0.95,5,8} = 4.82$

Where: Y_1 is a total reducing power (mg GAE g⁻¹)

Y_2 is a DPPH antioxidant activity ($\mu\text{mol Trolox g}^{-1}$)

Y_3 is an ABTS antioxidant activity ($\mu\text{mol Trolox g}^{-1}$)

x_1 is an ethanol concentration in %

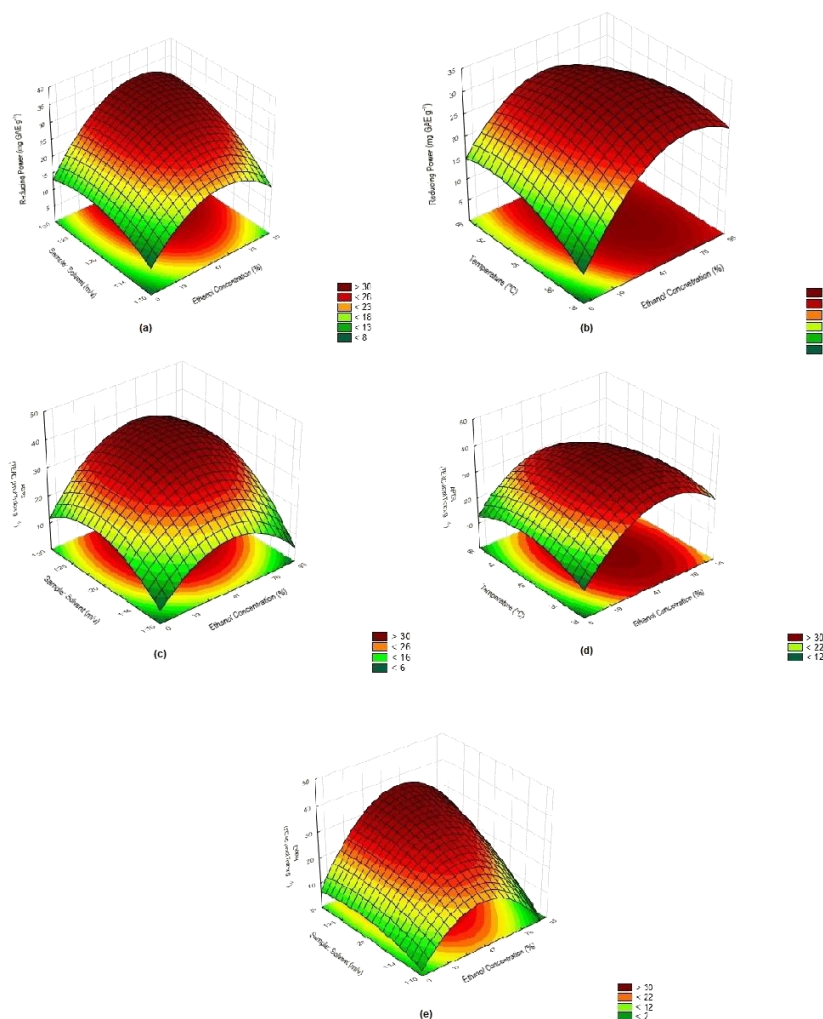
x_2 is a proportion sample: solvent in m/v

x_3 is a temperature in °C

The graphs of the response surfaces presented, in agreement with ANOVA and effects analyzes, allowed to conclude that for the three variables there was an optimal range of extraction and again the interaction was not significant. For reducing power response, the optimum extraction range was close to the central point (47%, 45°C and 1:20), with an ethanol concentration of 25-75%, a ratio of 1:15-1:30 and a temperature of 35-55°C. It was observed that ethanol concentration between 45-85%, a higher sample: solvent ratio (1:20-1:30) and temperatures between 36-54 °C can be used to extraction of active compounds. For DPPH the optimum range was with an ethanol concentration of

20-75%, a ratio of 1:20-1:30 and a temperature of 35-55 °C. For ABTS the optimum extraction range was with ethanol concentration of 25-85%, ratio of 1:15-1:30 and temperature of 35-55°C, and it was verified that higher sample: solvent ratios (1:20-1:30), wide ethanol concentrations (30-80%) and temperatures (36-54 °C) can be used (Figure 2). Sousa, Vieira, and Lima (2011) evaluated the antioxidant capacity by ABTS in acerola residues and obtained values around 0.518-0.743 mmol Trolox g⁻¹.

Figure 2. Surface response of acerola seed optimization for reducing power (a, b), ABTS (c, d) and DPPH (e).



(a) Ethanol concentration x Sample: solvent for reducing power; **(b)** Ethanol concentration x Temperature for reducing power; **(c)** Ethanol concentration x Sample: solvent for ABTS; **(d)** Ethanol concentration x Temperature for ABTS; **(e)** Ethanol concentration x Sample: solvent for DPPH.

3.2 Sample characterization

The chemical composition of acerola presents differences, which are due to factors such as the cultivar (genetic differences), environmental conditions of cultivation region (rainfall, temperature, altitude, fertilization, irrigation, occurrence of pests and diseases) and maturation process. The vitamin C content, pH, soluble solids content (SST), color, weight and size of the fruits are characteristics attributed to the quality of the fruit, which are also influenced by the mentioned factors, and therefore present differences when evaluating the fruit (Vendramini & Trugo, 2000; Kawaguchi, Tanabe, & Nagamine, 2007; Nogueira et al., 2002; Souza et al., 2006). Vendramini and Trugo (2000) verified low alteration in pH, increase in titratable acidity (TA), sugars and soluble solids, and decrease of vitamin C contents with maturation. Therefore, when analyzing the physicochemical characterization of pulps and ripe fruits, it was observed that the samples differed in relation to the coloring parameters, showing differences in the stages of maturation of raw materials and quality and quantity of chemical composition of the compounds responsible for the coloring of the fruits, such as carotenoids, anthocyanins and flavonoids.

When evaluated the instrumental color, the commercial pulp samples presented higher results of the parameters of luminosity color (L^*) (55.44), b^* (45.64), Hue (59.21) and Chroma (53.13) being statistically different from the other samples. Only for a^* parameter, the mature fruit had the highest result (30.32), differing statistically from the others. This is mainly due to differences in crop location and genetic variety, but also to the different stages of maturation, harvesting season, and climatic and soil conditions (Table 1). For the color parameters, studies found L^* values between 23.8-44.07, a^* between 7.84-37.16, b^* between 20.05-25.06, Hue between 19.6-35.25 and Chroma between 33.2-48.23 (Adriano, Leonel, & Evangelista, 2011; Canuto et al., 2010; Lima et al., 2014; Mercali et al., 2014; Jaeschke, Marczak, & Mercali, 2016).

Also regarding the fruit and vegetable instrumental color, studies have shown high correlation between color parameters and bioactive compounds. For example, it was observed correlation between color parameters and the presence of carotenoids, anthocyanins and other polyphenols, and chlorophyll (Sant'anna et al., 2013; Meléndez-Martínez et al., 2007; Spada et al., 2012; Jiménez-Aguilar et al., 2011; Larrauri, Rupérez, & Saura-Calixto, 1997; Koca, Karadeniz, & Burdulu, 2006). The color is also an important quality indicator when it is desired to evaluate the effects of the processing carried out on a fruit, being observed for acerola, that the parameter L^* is associated to non-enzymatic darkening phenomena and the decrease of ascorbic acid, the parameter a^* related to the anthocyanin content and, finally, the parameter b^* related to the yellow carotenoid contents (Mercali et al., 2014).

Table 5. Results of Color parameters (L, a*, b*, Hue e Chroma) in lyophilized pulps and mature fruit of acerola

Sample	Color				
	L	a*	b*	Hue	Chroma
Comercial Pulp	55.44 ± 0.13 ^a	27.20 ± 0.04 ^b	45.64 ± 0.02 ^a	59.21 ± 0.02 ^a	53.13 ± 0.04 ^a
Pulp	49.74 ± 0.08 ^b	19.63 ± 0.02 ^c	28.19 ± 0.02 ^b	55.15 ± 0.01 ^b	34.35 ± 0.03 ^c
Acerola Fruit	45.74 ± 0.01 ^c	30.32 ± 0.04 ^a	27.09 ± 0.03 ^c	41.78 ± 0.07 ^c	40.66 ± 0.01 ^b

Triplicate mean ± standard deviation (SD)

Same letters in column not differ significantly for $p \leq 0.05$ ($\alpha = 5\%$)

Regarding the other physicochemical parameters evaluated, pH and soluble solids content (SST) were significantly higher in the pulp, differing statistically from the others. Similar situation was observed in the results of titratable acidity (TA), however the pulp sample did not differ from the mature fruit. Higher ratio values were observed in the commercial pulp, which was statistically higher than in the other samples (Table 2). Some investigations of physicochemical characterization of acerolas found pH values around 2.8-3.76, soluble solids content ranging from 3.5-11.3 °Brix, titratable acidity between 0.53-1.52 g citric acid 100 g⁻¹ (%), and ratio (relation between soluble solids content with the titratable acidity – SST/TA) ranging from 2.41-8.31 (Adriano, Leonel, & Evangelista, 2011; Aquino, Móes, & Castro, 2011; Canuto et al., 2010; Lima et al., 2014; Mercali et al., 2014; Moura et al., 2007; Santos et al., 2012; Vendramini & Trugo, 2000).

Table 6. Results of Physicochemical parameters of pH, titratable acidity (TA), content of soluble solids total (SST) and ratio (SST/TA) in lyophilized pulps and mature fruit of acerola

Sample	pH	TA	SST	Ratio
Comercial Pulp	3.36 ± 0.00 ^c	0.93 ± 0.00 ^b	6.93 ± 0.05 ^b	7.47 ± 0.06 ^a
Pulp	3.46 ± 0.01 ^a	0.99 ± 0.00 ^a	7.27 ± 0.05 ^a	7.30 ± 0.03 ^b
Acerola Fruit	3.41 ± 0.02 ^b	1.00 ± 0.01 ^a	5.77 ± 0.05 ^c	5.77 ± 0.03 ^c

Triplicate mean ± standard deviation (SD)

Same letters in column not differ significantly for $p \leq 0.05$ ($\alpha = 5\%$)

3.3 Extract characterization

Carrying out the antioxidant characterization and evaluating the reducing power and the antioxidant capacity of the extracts it was verified that only the ascorbic acid content did not differ between the two samples of acerola pulp studied. For the reducing power, ABTS, FRAP and total anthocyanins by the two methods (differential pH and HPLC) the commercial pulp presented higher results. For DPPH and ORAC (total, hydrophilic and lipophilic) the pulp presented values significantly higher in comparison to commercial pulp.

When evaluating the reducing power, whose results were 142.04 and 178.78 mg GAE g⁻¹ for pulp and commercial pulp respectively, it can be verified high results in the studies, which ranged from 247.62-1679 mg GAE 100 g⁻¹ for acerola (pulp and fruits) (Batiston et al., 2013; Hassimotto, Genovese, & Lajolo, 2005; Kuskoski et al., 2005, 2006a; Mercali et al., 2014; Mezadri et al., 2008; Rufino et al., 2010; Sousa, Vieira, & Lima, 2011; Sousa et al., 2011), being the high amplitude

observed explained by the different genotype varieties (Lima et al., 2005) and by environmental factors such as light and temperature (Macheix, Fleurit, & Billot, 1990). Besides the mentioned aspects, the content of phenolics is strongly influenced by the degree of maturation of the fruit (Righetto, Netto, & Carraro, 2005).

As far as quantification and identification of anthocyanin and non-anthocyanin phenolic compounds were concerned, it was verified in the present study significantly higher values of chlorogenic and ferulic phenolic acids in the pulp, and of p-coumaric phenolic acid, and the anthocyanins cyanidin-3-rhamnoside and pelargonidin-3-rhamnoside in commercial pulp. Some studies such as Mezadri (2005) and Mezadri et al. (2008) have identified the following compounds in acerola: furfural, procyanidin B1, chlorogenic acid, epigallocatechin gallate, epicatechin and rutin. Hanamura, Hagiwara, and Kawagishi (2005) identified two anthocyanins cyanidin-3-rhamnoside and pelargonidin-3-rhamnoside and a flavonol quercetin. Hanamura, Uchida, and Aoki (2008) in addition to the anthocyanin pigments cyanidin-3-rhamnoside and pelargonidin 3-rhamnoside have also identified ferulic, p-coumaric and chlorogenic phenolic acids. Vendramini and Trugo (2004) also detected phenolic acids and flavonoids, being identified the phenolic acids p-coumaric, ferulic, caffeic and chlorogenic, the flavonoids kaempferol and quercetin, and the anthocyanins malvidine 3,5-diglycosylated, cyanidin 3- glycosylated and pelargonidin aglycone ("free").

In relation to the class of anthocyanic phenolic pigments, the results obtained for pulp and commercial pulp were 0.06 and 0.08 mg cyanidin-3-glycoside g⁻¹ respectively. The studies revealed averages between 2.16-59.74 mg cyanidin-3-glycoside 100 g⁻¹ and also observed a relationship between this class of compounds and the coloration developed in the fruit, and it was verified that samples with a more intense red color are those with the highest concentrations of these compounds (De Rosso et al., 2008; De Rosso & Mercadante, 2007; Düsman et al., 2014; Kuskoski et al., 2005; 2006a; Lima et al., 2003; 2000; Mercali et al., 2013; 2014; Mezadri, 2005; Mezadri et al., 2008; Vendramini & Trugo, 2004). De Rosso et al. (2008) also carried out a study to identify phenolic compounds, and through the use of the HPLC-MS/MS analytical technique it was possible to identify the presence of anthocyanins cyanidin 3-rhamnoside, pelargonidin 3-rhamnoside and its free aglycone forms cyanidin and pelargonidin, which accounted for 76-78%, 13-16%, 6-8% and 2-3% of total anthocyanin content in acerola, respectively.

Concerning the antioxidant capacity, the DPPH results for pulp and commercial pulp were 372.14 and 257.42 µmol Trolox g⁻¹ and the results for ABTS were 538.62 and 789.60 µmol Trolox g⁻¹. Studies have revealed averages ranging from 38.31 to 125.66 µmol Trolox g⁻¹ for DPPH and 32.93-119.97 µmol Trolox g⁻¹ for ABTS (Kuskoski et al., 2005; 2006a; Mezadri, 2005; Mezadri et al., 2008; Oliveira et al., 2012; Rufino et al., 2010; Souza et al., 2014). Rufino et al. (2010) in their study of the antioxidant capacity of tropical fruits reported that the ABTS method is generally more indicated to evaluate the antioxidant activity of hydrophilic compounds, whereas the DPPH method quantifies the antioxidant capacity of both hydrophilic and lipophilic compounds, being widely used for aqueous and/or organic extracts. For the ORAC and FRAP antioxidant capacity evaluation methods, the results for pulp and commercial pulp were respectively 202.27 and 155.88 µmol Trolox g⁻¹ for the ORAC method and 451.77 and 705.05 µmol Trolox g⁻¹ for the FRAP method. In the literature, values ranging

from 34.58-173.77 for ORAC and 334-341.19 $\mu\text{mol Trolox g}^{-1}$ for FRAP was found (Kuskoski et al., 2006b; Mezdari et al., 2008; Mezdari, Pérez-Gálvez, & Hornero-Méndez, 2005; Rezende, Nogueira, & Narain, 2017; Seraglio et al., 2018).

By analyzing and comparing antioxidant capacity and the presence of bioactive compounds it is a consensus among the studies the significant contribution of these substances to the high antioxidant capacity verified in fruits and vegetables in general, and therefore, a strong correlation between the presence and contents of these phytochemicals as phenolics, vitamin C and carotenoids and the antioxidant capacity (DPPH, ABTS, ORAC and FRAP) methods were reported and observed by researches (Delva & Goodrich, 2010; Mezdari, Pérez-Gálvez, & Hornero-Méndez, 2005; Rufino et al., 2010).

As regards the presence of vitamin C in acerola, it is verified that this is of great importance and relevance in the chemical composition of the fruit, being one of the main bioactive compounds of the fruit. Studies have indicated that vitamin C content in acerola can vary from 0.8 to 3.5% in fresh fruit (Alves, Chitarra, & Chitarra, 1995; Alves et al., 1999; 2008). Therefore, the vitamin C contents obtained were 61.23 and 64.31 mg g^{-1} for pulp and commercial pulp. The literature indicates a wide range of results, which varied around 470-4827 $\text{mg ascorbic acid } 100 \text{ g}^{-1}$ (Gomes et al., 2000; Mezdari et al., 2008; Oliveira et al., 1999; Rufino et al., 2010; Vendramini & Trugo, 2000; Santos et al., 1999). The differences observed in vitamin C content in acerolas and in fruits rich in this phytochemical in general are due to the great instability of this and mainly to the factors of cultivation (soil, climate, variety), degree of maturation, and physicochemical characteristics, such as weight, shape and size of the fruit (Cardoso et al., 2011; Lima et al., 2005; Matsuura et al., 2001; Nogueira et al., 2002; Soares et al., 2001; Vendramini & Trugo, 2000).

Table 7. Antioxidant activity (Reducing Power, DPPH, ABTS, ORAC and FRAP), anthocyanins and phenolic and ascorbic acids quantification in acerola pulps (pulp and comercial pulp).

	For g of sample		For ml of extract	
	Pulp	Comercial Pulp	Pulp	Comercial Pulp
Reducing Power (mg GAE)	142.04±2.97 ^b	178.68±4.31 ^a	7.18±0.15 ^b	9.55±0.45 ^a
Chlorogenic Ácid (µg)	278.43±0.68 ^a	161.10±1.66 ^b	13.92±0.03 ^a	8.06±0.08 ^b
Ferulic Acid (µg)	60.99±0.15 ^a	34.05±0.38 ^b	3.05±0.01 ^a	1.70±0.02 ^b
p-Coumaric Acid (µg)	62.59±4.77 ^b	90.41±2.30 ^a	3.13±0.48 ^b	4.57±0.12 ^a
DPPH (TEAC µmol Trolox)	372.14±3.19 ^a	257.42±1.28 ^b	18.61±0.16 ^a	12.87±0.06 ^b
ABTS (TEAC µmol Trolox)	538.62±4.37 ^b	789.60±8.05 ^a	27.15±0.44 ^b	39.48±0.40 ^a
ORAC-FL-Total (TEAC µmol Trolox)	202.27±3.08 ^a	155.88±4.46 ^b	10.35±0.43 ^a	8.11±0.59 ^b
ORAC-FL-H (TEAC µmol Trolox)	198.43±3.04 ^a	154.00±4.46 ^b	10.15±0.43 ^a	8.02±0.59 ^b
ORAC-FL-L(TEAC µmol Trolox)	3.88±0.07 ^a	1.87±0.01 ^b	0.19±0.00 ^a	0.09±0.00 ^b
FRAP (TEAC µmol Trolox)	451.77±1.40 ^b	705.05±2.20 ^a	22.59±0.07 ^b	35.36±0.22 ^a
Ascorbic Acid (Vitamin C) (mg AA)	61.23±2.51 ^a	64.31±3.52 ^a	2.94±0.25 ^a	3.50±0.40 ^a
Total Anthocyanins (mg cyanidin-3-Glucoside)	0.06±0.01 ^b	0.08±0.00 ^a	0.06±0.01 ^b	0.08±0.00 ^a
Total Anthocyanins (mg)	10.67±0.10 ^b	22.27±0.09 ^a	0.54±0.01 ^b	1.11±0.00 ^a
Cyanidin-3-Rhamnoside (mg)	9.90±0.09 ^b	18.06±0.13 ^a	0.50±0.00 ^b	0.90±0.01 ^a
Pelargonidin-3-Rhamnoside (mg)	0.77±0.01 ^b	4.21±0.04 ^a	0.04±0.00 ^b	0.21±0.00 ^a

Results express for ml of extract or for g of sample in dry weight (DW)

Triplicate mean ± standard deviation (SD)

Same letters in lines not differ significantly for $p \leq 0.05$ ($\alpha = 5\%$)

GAE – Gallic Acid Equivalent

TEAC – Trolox Equivalent Antioxidant Capacity

AA – Ascorbic Acid

ORAC-FL-H – Hydrophilic ORAC

ORAC-FL-L – Lipophilic ORAC

3.4 Oxidative stability

Analytical methods developed to evaluate the evolution of lipid oxidation in food emulsions are usually based on quantifying different lipid oxidation products that develop or are produced during oxidative lipid process steps (Jacobsen, 1999). Studies suggest that for margarines, mayonnaise, salad dressings, spreads and dairy products the rancidification level of the oils present in these products is evaluated mainly by peroxide index, conjugated dienes, anisidine or p-anisidine, and total carbonyl content, with the peroxide index being the most recommended for evaluating the initial oxidation stage and the total amount of carbonyl compounds and the anisidine value most recommended to evaluate the final stage (Jacobsen, 1999; Li Hsieh & Regenstien, 1992).

The different existing methodologies quantify different oxidation products, such as peroxides and hydroperoxides, dienes and dienoic acids, among others, and the recommendations based on the use of one or other methodology are based on the fact that the oils have different compositions in fatty acids and with that different types and levels of oxidation products are formed, which explains why some methods are more appropriate than others depending on the type of oil predominant in the food or matrix in study (Bosset & Fluckiger, 1989; Frankel, 1998; Li Hsieh & Regenstien, 1991; 1992).

According to the literature reports, the schaal oven test and oxidative stability evaluation performed by means of quantitative analysis of oxidation products formed in oils and emulsified lipid

matrix products using the peroxide index and UV Absorptivity methods, were chosen as analytical procedures more adequate to the evaluation of lipid oxidation in emulsified lipid systems. With this, the use of antioxidants (natural or synthetic), as well as the amounts and form of application, the choice of oil type (sunflower, canola, corn) and storage time were also studied since they are factors of great importance with regard to the quality and *shelf life* of oils and fats and lipid-based food products.

The literature also reports that in emulsified lipid systems, one of the main triggers of lipid oxidation is the decomposition of hydroperoxides in free radicals promoted by factors such as oxygen, temperature, light and, mainly, metallic ions, such as iron, for example (McClements & Decker, 2000). However, as these unstable substances and the major oxidation promoters agents (metals) are located in the surface regions of droplets/micelles (interface of the emulsion) and in the aqueous phase, it has been suggested that oxidation reactions occur preferentially in these regions of the system (Nuchi, McClements, & Decker 2001; Mancuso, McClements, & Decker, 1999; Decker et al., 2005; Frankel, 1996, Frankel & Meyer, 2000; Heins et al., 2007).

In addition to this, there are some factors that influence lipid oxidation. Studies have reported that storage temperature is a significant factor for the oxidative stability of lipid systems (oils and emulsions), as an increase in oxidative deterioration was observed with increasing storage temperature (Dimakou et al., 2007). Another important factor to be considered is the concentration of the oil phase of an emulsion, which was also observed an increase in the oxidative reactions with the decrease of the oil phase concentration (Kiokias & Oreopoulou, 2006; Osborn & Akoh, 2004). The size of a droplet of an emulsion in this sense is also relevant in lipid oxidation, since studies have shown that the rate of lipid oxidation is increased as the droplet size decreases, this is because there is a larger surface area of the oil droplet exposed to the aqueous phase and oxidizing agents, which are concentrated on the surface of the micelle (interface between the phases of the emulsion) and in the aqueous phase of the system. In view of this, it can be seen that these regions of the emulsion are the most important in terms of oxidative stability, which oxidation phenomena occur more quickly and significantly (McClements & Decker, 2000; Osborn & Akoh, 2004; Roozen, Frankel, & Kinsella, 1994).

The presence of metal ions in emulsions was another parameter studied, being verified in the studies that salts containing ions such as sodium chloride and potassium (NaCl, KCl) and iron ions present pro-oxidant behavior (Chen et al., 2012; Mei et al., 1998). In view of this, it can be concluded that lipid-based foods such as mayonnaises and butter, products with significant salt concentrations in their formulations, present as main factor of deterioration and decrease of the *shelf life* the lipid oxidation, as they present great susceptibility to the lipid oxidation phenomena, thus confirming the pro-oxidant potential of metallic ions in general, such as sodium, potassium and iron (Cui et al., 2016; Heshmati, Vahidinia, & Salehi, 2014; Khaniki et al., 2007).

In this sense, when performing the analyses to evaluate the evolution of lipid oxidation, it was expected that the start of storage (day 0) presented a lower level of oxidation and consequently lower levels of oxidation products (peroxides and conjugated dienes), and that the ninth day and therefore the last period evaluated had higher levels of peroxides and conjugated dienes since it is known that the storage of emulsified oils and lipid products (for example, mayonnaises and margarines) exposes the lipids to the various pro-oxidants agents previously mentioned as oxygen, light, metal ions and

others, allied to the fact that antioxidants, both added and naturally present, have already been consumed. When carrying out the analyzes, these observations were confirmed, being only the level of peroxide in treatments with corn oil the exception; in treatments with corn oil, the sixth day of storage was the one that presented higher levels of peroxide (Table 8).

Kiokias and Varzakas (2014) reported that with the storage time emulsions with cottonseed and sunflower oil were the ones that obtained the highest increase in the oxidation parameters and the emulsions of corn and olive oil, which obtained lower oxidation levels at the end of storage. The results obtained in the study were mainly explained by the composition in fatty acids of vegetable oils, since there is a consensus that oils with higher concentrations of polyunsaturated fatty acids of the linoleic type ($C_{18:2}$, fatty acid with two unsaturations or double bonds), are more susceptible to oxidation and an even higher rate of oxidative reactions is verified in these oils.

Table 8. Hydroperoxide content (mmol CHP L⁻¹) and UV Absorptivity (Conjugated Dienes) during storage at 40°C (0, 3, 6, 9 days) of lipid emulsions formulated with sunflower, canola and corn oil, added of natural antioxidant extracts from pulp and fruit of acerola and synthetic antioxidant BHA/BHT.

Period (days)	Hydroperoxide content (mmol CHP L ⁻¹)			UV Absorptivity (Conjugated Dienes)		
	Sunflower	Canola	Corn	Sunflower	Canola	Corn
0	0.94±0.13 d	0.93±0.19 d	0.16±0.07 d	1.71±0.50 c	3.04±0.36 c	2.31±0.11 c
3	1.21±0.27 c	1.10±0.22 c	0.28±0.09 b	2.18±0.16 b	3.15±0.30 b	2.38±0.11 b
6	2.02±0.53 b	1.57±0.19 b	0.38±0.14 a	2.30±0.13 a	3.09±0.24 bc	2.42±0.10 b
9	2.22±0.57 a	1.70±0.50 a	0.25± 0.17 c	2.34±0.34 a	3.28±0.20 a	2.49±0.16 a

Mean of all treatments ± standard deviation (SD) (each treatment in triplicate)
 Same letters in columns not differ significantly for p≤0.05 (α = 5%)
 CHP – Cumene Hydroperoxide

it was verified that one of the main influencing factors is the physicochemical behavior and consequently the partitioning of these compounds in the system/micelle, which is mainly dependent on physical structure and physicochemical characteristics (polarity, reactivity). As an example, antioxidants considered more hydrophilic/polar are less effective in their activity as antioxidants in emulsions (complex lipid systems) while more lipophilic/apolar compounds are less effective in bulk oils (simple lipid systems) (Frankel, 1996). This difference in the efficiency of the compounds was explained and described by Frankel (1996) and Porter (1993) in the so-called "Polar antioxidant paradox", in which it is reported that an antioxidant is only effective and efficient in reducing the chain reactions that lead to oxidation and decrease in the quality and *shelf life* of lipid food products when it is concentrated in more reactive and unstable sites, that is, of a higher concentration of lipid hydroperoxides and oxidation initiators (oxygen, metals, free radicals) and therefore where hydroperoxides are more easily decomposed into free radicals by oxidative factors. In this sense, it is observed that the surface of the micelle (oil-water interface) consists of the most unstable and reactive region of a lipid system (Jacobsen, Meyer, & Adler-Nissen, 1998; 1999; Jacobsen, et al., 1999; Sasaki et al., 2010).

In view of the above, it was expected to quantify higher levels of lipid oxidation products and consequently higher levels of oxidation in control treatments, since in these treatments the lipids do

not present protection of their chemical structure by the antioxidant substances, and therefore lipid oxidation is given in greater degree and speed in these treatments with absence of antioxidants. Only for conjugated diene content in treatments with canola oil an exception was observed: for this oil the conjugated diene content was higher in the treatment with addition of 200 ppm synthetic antioxidant mixture (BHA + BHT) (Table 9).

Table 9. Hydroperoxide content (mmol CHP L⁻¹) and UV Absorptivity (% Conjugated Dienes) in lipid emulsions formulated with sunflower, canola and corn oils, added of extract from pulp and fruit of acerola and synthetic antioxidant BHA/BHT during nine days of schaal oven storage at 40°C.

Treatment	Hydroperoxide content (mmol CHP L ⁻¹)			UV Absorptivity (Conjugated Dienes)		
	Sunflower	Canola	Corn	Sunflower	Canola	Corn
1 – Control	2.10±0.96 a	1.58±0.38 a	0.52±0.15 a	2.42±0.45 a	2.88±0.38 d	2.52±0.17 a
2 - Acerola 200 ppm	1.25±0.35 d	1.33±0.40 b	0.24±0.08 c	2.11±0.07 c	3.20±0.48 bc	2.44±0.12 ab
3 - Acerola 400 ppm	1.19±0.19 d	1.12±0.49 d	0.16±0.06 e	2.20±0.11 bc	3.07±0.24 c	2.27±0.08 d
4 - Acerola Pulp 200 ppm	1.82±0.51 b	1.53±0.83 a	0.18±0.07 de	2.10±0.15 c	3.11±0.20 c	2.44±0.11 ab
5 - Acerola Pulp 400 ppm	1.69±0.73 c	1.06±0.18 d	0.22±0.07 c	1.94±0.56 d	3.06±0.21 c	2.34±0.07 cd
6 - BHA+BHT (100 +100 ppm)	1.22±0.47 d	1.30±0.25 bc	0.31±0.18 b	1.83±0.66 d	3.41±0.09 a	2.49±0.10 a
7 - BHA 200 ppm	1.59±0.58 c	1.29±0.16 c	0.22±0.06 cd	2.24±0.30 b	3.27±0.10 ab	2.39±0.05 b
8 - BHT 200 ppm	1.89±0.82 b	1.40±0.29 b	0.26±0.11 c	2.21±0.25 bc	3.13±0.11 bc	2.31±0.15 cd

Mean of all periods ± standard deviation (SD) (each period in triplicate)

Same letters in columns not differ significantly for p≤0.05 (α = 5%)

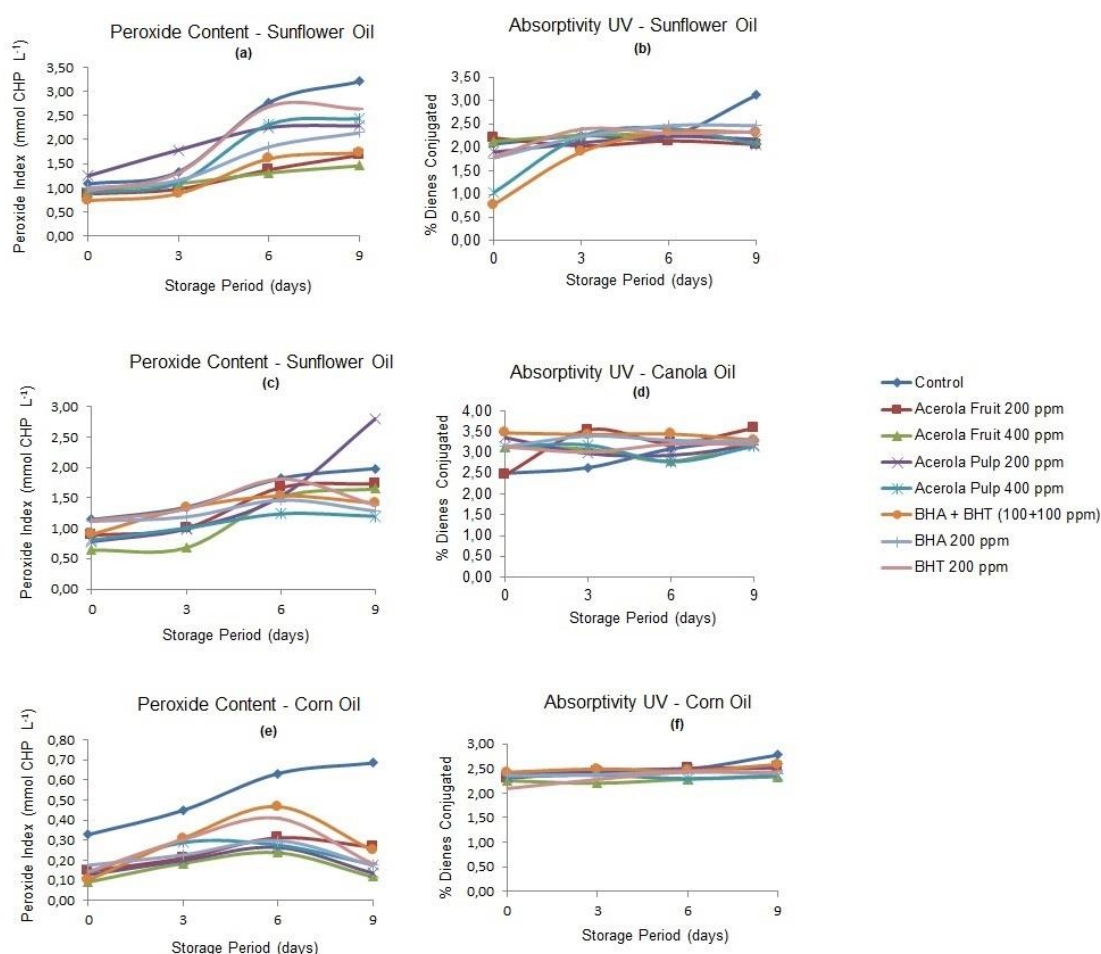
CHP – Cumene Hydroperoxide

The evaluation of oxidative stability allowed to observe that with the storage time, there was an increase in the hydroperoxide content and the absorptivity (conjugated dienes). Therefore, lower levels of these oxidation products were obtained at the beginning of storage (0 days) and higher levels in the last storage period (9 days). For hydroperoxide content, all the periods evaluated differed statistically from each other. For the conjugated diene levels, the intermediate periods (days 3 and 6) did not differ statistically from each other.

Regarding the treatments, it can be observed that the control treatment with sunflower oil was the one that presented higher value of hydroperoxide, being statistically different from the other treatments. In relation to the absorptivity, the treatment with canola oil with 200 ppm of the synthetic antioxidant mixture (BHA+BHT) showed the highest levels of conjugated dienes, not differing from the treatment with addition of 200 ppm of BHA (synthetic antioxidant). The treatments with sunflower oil added of 200 ppm of the synthetic antioxidant mixture (BHA+BHT) and added of 400 ppm of lyophilized acerola extract were those ones with the lowest absorbance values and did not differ statistically each other (Figure 3).

Caetano et al. (2011) observed that the concentration of peroxides and conjugated dienes increased in all treatments, reaching higher levels in control oil samples at the end of storage. It was also verified that the hydroethanolic extract was the most effective in controlling the lipid oxidation of soybean oil, and that ascorbic acid and BHT were the antioxidant substances with least effective in

lipid oxidation. Some other applications of natural extracts in oils and emulsions have shown high and effective antioxidant action of extracts obtained from natural sources in the protection of lipids from vegetable oils, such as garlic (1000 ppm), moringa oleifera (white acacia) (600 ppm), rice bran (600 ppm), rosemary (200-500 ppm), oregano (1000 ppm) and other natural products, which were effective in protecting vegetable oils (soybean, sunflower, safflower) of oxidation and significantly retarded the formation of peroxides, some of them being as efficient as synthetic ones (Almeida-Doria & Regitano-D'arce, 2000; Anwar et al., 2007; Chatha et al., 2006; Iqbal & Bhanger, 2007; Samotyja & Malecka, 2007; Suja et al., 2004).



b

Figure 3. Hydroperoxide content and UV Absorptivity of lipid emulsions formulated with sunflower (a) (b), canola (c) (d) and corn (e) (f) oils, added of extract from pulp and fruit of acerola and synthetic antioxidant BHA/BHT during nine days (0, 3, 6, 9 days) of schaal oven storage at 40°C

The analyzes of the emulsions with sunflower oil allowed to verify that the hydroperoxide content was statistically different for all the evaluated periods, being observed a significant increase, and, therefore, lower levels of peroxide at the beginning of storage (day 0) and higher levels in the last period (day 9) (Table 4). Absorptivity results also showed an increase in conjugated dienes over time. At the beginning of the storage (day 0), the result of the conjugated dienes was lower, being

statistically different from the other periods, higher result of this oxidation product was obtained at the end of storage (day 9), which did not differ only from the third period evaluated (day 6) (Table 4). The results obtained confirm and are in agreement with the expected and reported in the literature.

Regarding the treatments, that is, application of antioxidants, the control treatment was the one that presented higher levels of peroxide, differing statistically from the others. The treatment with the addition of 400 ppm of lyophilized acerola extract showed lower levels of peroxide, thus demonstrating the great antioxidant potential of natural sources (acerola) with respect to lipid oxidation. This treatment did not differ statistically from the other treatment added with acerola extract (200 ppm) and the treatment with 200 ppm of the synthetic antioxidant mixture (BHA+BHT) (Table 5). The control treatment was also the one that presented the highest levels of conjugated dienes, being this treatment statistically different from the others evaluated. In addition, the treatment of 200 ppm of the synthetic antioxidant mixture (BHA+BHT) showed the lowest levels of conjugated dienes, not differing only from the treatment with 400 ppm of lyophilized acerola pulp extract (Table 5).

A study with application of vegetable extracts in oils and lipid emulsions conducted by Abdalla and Roozen (1999) allowed to verify that the formation of primary oxidation products, whose main exponents are hydroperoxides, was high in both products (bulk oil and emulsion). However it occurred faster in bulk oil than in emulsions, since the increase of these compounds was significant from the 1st and 4th day of storage for bulk oils and emulsions respectively. It was also observed a lower rate of hydroperoxide formation in the samples with higher concentrations of vegetal extracts, for both bulk oils and emulsions. In spite of the sunflower oil and the emulsion prepared with this oil, it was verified that the sage extract was the most effective in the inhibition and reduction of the oxidation phenomena, which was proved by the low levels of primary and secondary compounds of oxidation quantified, significantly lower than the other extracts tested.

For the emulsions with canola oil, a statistical difference was observed in the peroxide index for all the periods evaluated, that is, a significant increase of this oxidation product with the storage time, and, therefore, lower levels were obtained at the beginning of the storage (day 0) and higher levels in the last period (day 9) (Table 9). Regarding the treatments studied, the control treatment (without antioxidants) was the one that presented higher levels of peroxide, only not statistically differing from the treatment with addition of 200 ppm of lyophilized acerola pulp extract. The treatment with the addition of 400 ppm of lyophilized acerola pulp extract showed lower peroxide levels, but did not differ statistically from the treatment of 400 ppm of lyophilized acerola extract. These results allow to observe and verify the great antioxidant potential of the natural sources (acerola) with respect to lipid oxidation, regardless of the way the raw material is available (pulp or whole fruit).

The absorptivity results demonstrated once again, increase in conjugated dienes over the time of storage. The storage time (day 0) showed a lower result of conjugated dienes, which did not differ statistically from the third storage period (day 6), at the end of the storage (day 9) the highest result of this oxidation product was obtained which differed statistically from the other periods. Intermediate storage periods (days 3 and 6) did not differ statistically from each other (Table 4). The results of the analyzes of corn oil emulsions allowed to verify statistical difference in the peroxide index for all evaluated periods, that is, a significant increase of this oxidation product was observed during the

storage, and, therefore, lower levels were obtained at the beginning of the storage (day 0) and higher levels in the last period (day 9). Regarding the treatments studied, the control treatment (without addition of antioxidants) was the one that presented higher levels of peroxide, differing statistically from the other treatments studied. The treatment with the addition of 400 ppm of lyophilized acerola extract showed lower levels of peroxide but did not differ statistically from the treatment of 200 ppm of lyophilized acerola pulp extract. Absorptivity results again showed an increase over storage time. The storage time (day 0) showed a lower result of conjugated dienes, which did not differ statistically from the third storage period (day 6), at the end of the storage (day 9) the highest result of this oxidation product was obtained which differed statistically from the other periods. Intermediate storage periods (days 3 and 6) did not differ statistically from each other (Table 4). The control treatment showed higher levels of conjugated dienes, which did not differ statistically from the treatments with addition of 200 ppm of the synthetic antioxidant mixture (BHA+BHT), with addition of 200 ppm of lyophilized acerola pulp extract, and with addition of 200 ppm lyophilized acerola extract. The results obtained for both oxidation products (peroxides and conjugated dienes) in emulsions with corn oil are in agreement with the expected results and with the results obtained for emulsions with sunflower and canola oil, both as regards the storage time and the treatments evaluated, being exception, the results of peroxide index for the storage time.

4. Conclusions

The experimental design was a relevant tool in the evaluation as well as the determination of the variables that demonstrated influence on the extraction process, which for both pulp and seed were the concentration of ethanol and sample: solvent ratio. By employing the experimental design for the optimization a clear reduction of variation and time was reached, along to show a possibility of industrial application of natural antioxidant extracts from acerola pulp, which proved to be a potential alternative as a natural antioxidant due to its high composition in antioxidants, mainly represented by the ascorbic acid and phenolic compounds.

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CHAPTER 3. SENSORIAL EVALUATION BY FLASH PROFILE AND GLOBAL ACCEPTANCE OF LIPID EMULSION (MAYONNAISE) ADDED OF ACEROLA ANTIOXIDANT

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Original paper submitted to “Food Quality and Preference”

Abstract

Descriptive techniques develop a sensorial profile of the food products performed by raising sensory attributes. This aim to create an identity for the product, explain consumer perceptions and preferences and inform about perception and sensory impact of a new product on consumers when changes are made to the formulation, process or packaging. The flash profile is a technique developed as a combination of the free-choice profile with the sorting ranking. Due to the importance of sensorial analysis in product development, the objective of this work was to perform sensorial evaluation (flash profile and acceptance) of mayonnaises added of acerola and synthetic antioxidants. The results showed that for the samples distribution was a greater approximation between the samples profiles, being the sample added of synthetic antioxidant the one with a more distinct sensorial description; assessors' distribution was close, demonstrating relative agreement. The assessors and samples variances were low, reflecting consensus among assessors and little differentiation between the samples. When evaluating the acceptance, addition of 400 ppm of acerola presents greater acceptance, differing statistically from the others. When correlating the profile and acceptance, there was a high consensus, low discriminative power and similar acceptance, confirmed by the low variances and proximity of distribution, which allows to conclude that the substitution of synthetic antioxidants by acerola does not cause damages to the sensorial quality, being little noticeable and presenting significant sensorial acceptance.

Keywords: Sensory analysis; Natural antioxidants; Acceptance; Flash profile; Consumers

1. Introduction

Sensory analysis with their different techniques have been developed since the decade of 1950, being a strong method for the food industry in the knowledge of consumers impressions and preferences. This is due the sensory evaluation methods are tools through which the industry can assess the quality (acceptance/preference), identify/describe attributes and develops descriptive profiles of foods, and, therefore, some methodologies are used to investigate the sensory properties and attributes of both traditional market products and new products. Sensory evaluation impacts decisions on changes in products such as formulation, process or package, or even ingredients substitutions, as well as to assist the *shelf life* determination in a food product.

Sensory profile became an important tool to be employed in new products development, as it allows getting information about preferences and consumer's needs, through the survey of sensory attributes, description and quantification of sensory differences of food products allowing to position and description of products in a sensory space of multiple sensory attributes, which should be relevant to the product group (Delarue & Sieffermann, 2004). The descriptive technique through descriptive information (sensory attributes) creates identities and profiles for foods, therefore it uses tasters free of affective (hedonic) judgments as a tool for evaluation of a product. However, because humans are not equally discriminating in sensory attributes, for some descriptive techniques there is a need for a trained panel assessors aimed at better approximation of the consumers' impressions (Delarue & Sieffermann, 2004; Liu et al., 2016).

In order to improve the sensory analysis techniques, Sieffermann (2000, 2002) has developed a methodology described and presented as a combination between free choice profiling and an ordination technique (ranking) called Flash Profile. As a descriptive and comparative method, it is possible to make comparisons among products and to evaluate the product as a whole, through the classification of these in discriminant attributes, which should preferably emphasize sensory differences and describe both the products and the differences between them. It was developed to be a more flexible method and fast identification of a position and sensory profile of products. Also presents a greater advantage of time saving compared to the conventional descriptive profile, given its foundations in the free choice profile (Williams & Langron, 1984), allowing the assessors to use their own list of attributes, which makes it the fastest and with fewer constraints of consensus and concept alignment, finally reduces the need for a training with the panel of tasters, as well as the single-step accomplishment of the familiarization and survey phases of product attributes, through simultaneous access to the judges of all the samples (O'mahony, 1991).

This work aimed to evaluate the performance of lyophilized pulp and whole fruits extracts from acerola in a lipid base product (a mayonnaise) as a substitute for synthetic antioxidants. The sensorial evaluation was carried out by means of two methodologies, one of the description and sensory profile identification, a Flash profile, and other of acceptance, a global acceptance test.

2. Material and Methods

2.1 Emulsion preparation

Six formulations for sensory evaluation were prepared according to Di Matia and collaborators (2015) with adaptations. The commercial pulps of acerola were provided by the Junqueirópolis Agricultural Cooperative (COOPAJ), located in the city of Junqueirópolis/SP. The acerola fruits were acquired in a local market (Spoon Superalimentos) in the city of Porto, in Portugal. All the mayonnaise was prepared in the same way, with differences only in the concentration and type of antioxidant used, being the base formulation (control) made by blender pasteurized whole egg (250 ml), salt (3.75 mg) and vinegar (25 ml), and finally was added the refined sunflower oil (550 ml) sharp of wire to get the consistency. From it, there were five changes being: addition of 200 ppm lyophilized pulp (165 mg), addition of 400 ppm lyophilized pulp (330 mg), addition of 200 ppm lyophilized acerola (165 mg), addition of 400 ppm lyophilized acerola (330 mg) and finally added to 200 ppm mixture of synthetic antioxidants BHA and BHT (100 ppm of each; 82.5 mg of BHA and 82.5 mg of BHT). The samples were placed in airtight glass containers and then chilled (8-10°C) until the moment of analyses.

2.2 Sensory panels characterization

Sensory analysis, profile (Flash Profile) and acceptance were performed with assessors panel familiar with sensory evaluation tests. The sensory profile was performed with 14 assessors, aged between 24 and 47 years, 6 men and 8 women. The acceptance was evaluated by 60 assessors, 43 women and 17 men, aged between 21 and 54 years (Table 1).

Table 1. Characterization of assessors' panels

Characteristic of assessors panel	Flash Profile Panel	Acceptance Panel
Number of Assessors	14	60
Gender		
Female (F)	6 (43%)	43 (72%)
Male (M)	8 (57%)	17 (28%)
Age		
Minimum	24	21
Maximum	47	54
Mean \pm SD	33.5 \pm 6.7	34.6 \pm 8.6

Mean \pm standard deviation (SD)

2.3 Flash Profile

A descriptive sensory analysis of the type Flash Profile (Dairou & Sieffermann, 2002; Delarue & Sieffermann, 2004) was carried out with the six mayonnaise formulations being the samples presented

at the same time. In the first session, the attribute survey stage was carried out, in which the assessors were asked to establish discriminatory appearance, odor, texture, and taste attributes for the sample group. At the end of the session, the attributes raised were organized and for each assessor a list of the attributes was made, along with the individual lists a global list of attributes with all the attributes raised by the panel was made. In a second session, the two lists were presented, and the assessors were asked to compare their individual lists with the global list and to define the final list of attributes to be evaluated. The third session consisted in ordering the samples on a scale of 1 to 9, with 1 being the lowest intensity for attribute and 9 the highest intensity for the attributes defined in the previous steps. The results were analyzed with the statistical analysis GPA (Generalized Procrustes Analysis) (Gower, 1975) in XLSTAT software (XLSTAT, New York, NY, USA, 2013) as a way to obtain consensus between the sensorial maps of the assessors, to harmonize and normalize the data of the matrices of attributes of each assessor.

2.4 Global Acceptance test

The acceptance was performed with sequential presentation (monadic) of the samples, and the assessors were asked to quantify the magnitude or degree of acceptance of each of the products, using a structured hedonic scale of nine points (1 = dislike extremely, 5 = did not like or dislike, 9 = like extremely) (Stone & Sidel 1998). The results were analyzed by Wilcoxon test with 95% confidence interval in XLSTAT software (XLSTAT, New York, NY, USA, 2013).

2.5 Acerola Antioxidant characterization

The reducing power in acerola antioxidant was performed by the Folin-Ciocalteu method according to Singleton, Orthofer and Lamuela (1999), which uses gallic acid as standard and reads in spectrophotometer at 765 nm. The results were expressed in milligrams of gallic acid equivalent per ml of extract (mg GAE ml⁻¹). The antioxidant activity was evaluated by DPPH and ABTS methods, with Trolox standard (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and results expressed in Trolox equivalent antioxidant capacity (TEAC) per ml of sample. The method of evaluation of antioxidant activity by the DPPH was conducted according to methodology of Brand-Williams, Cuvelier and Berset (1995) adapted by Kim and collaborators (2002), which is based on the ability of antioxidant in reducing the oxidized DPPH radical (2,2-diphenyl-1-picrylhydrazyl) by means of hydrogen donation, being this reaction evaluated by discoloration and radical absorbance reading in spectrophotometer at a wavelength of 515 nm after 45 minutes of reaction. The ABTS method used was described by Re and collaborators (1999) and modified by Kuskoski and collaborators (2004). In this essay the antioxidant donates hydrogen atoms to reduced radical ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)), promoting your discoloration that is quantified in spectrophotometer with absorbance reading in 734 nm 6 minutes after reaction.

3. Results and Discussion

3.1 Antioxidant characterization

The results of the characterization of the acerola antioxidant samples are described below (Table 2). When evaluated the antioxidant activity by the total reducing power and antioxidant capacity (DPPH and ABTS), it was verified that the results demonstrated a relation about antioxidant capacity and presence of bioactive compounds (resulted present elsewhere). A strong correlation between the presence and contents of these phytochemicals as phenolics, vitamin C and the antioxidant capacity (DPPH, ABTS) methods were reported by the literature (Delva & Goodrich, 2010; Mezadri et al., 2008; Rufino et al., 2010).

Table 2. Antioxidant activity (Reducing Power, DPPH, ABTS) in comercial acerola pulp and acerola fruit

	Fruit	Comercial Pulp
Reducing Power (TPC - Total Phenolic Compounds) (mg GAE ml ⁻¹)	5.48±0.10 ^b	9.55±0.45 ^a
DPPH (TEAC µmol Trolox ml ⁻¹)	13.92±0.21 ^a	12.87±0.06 ^b
ABTS (TEAC µmol Trolox ml ⁻¹)	31.36±0.032 ^b	39.48±0.40 ^a

Results express for ml of extract

Triplicate mean ± standard deviation (SD)

Same letters in lines not differ significantly for p≤0.05 (α = 5%)

GAE – Gallic Acid Equivalent

TEAC – Trolox Equivalent Antioxidant Capacity

3.2 Flash Profile

The flash profile was initially applied to evaluated dairy products (yoghurts and cheeses) (Delarue & Sieffermann, 2004) and red fruit jams (Dairou & Sieffermann, 2002). This modern method, with regard to the type of panel to be used, has already been applied in studies with trained panels (6-12 trained or semi-trained assessors) (Albert et al., 2011; Moussaoui & Varela, 2010; Tarea, Cuvelier & Sieffermann, 2007) and with consumer panels (20-40 consumers) (Lassoued et al., 2008; Moussaoui & Varela, 2010; VEINAND et al., 2011). In addition, it has been used in studies with commercial apple and pear purée (Tarea, Cuvelier & Sieffermann, 2007), for the perception of taste and odor of bread (Poinot et al., 2007), and with jellies (Blancher et al., 2007), bread (Lassoued et al., 2008) e wines (Perrin et al., 2008).

In the study with dairy products, the authors could observe that for strawberry yoghurt, very similar results were obtained for the descriptive profiles obtained by different descriptive sensorial methods. On the other hand, the sensorial positioning of the fresh apricot cheeses showed little differentiation between the methods. For both sets of products, Flash Profile was slightly more discriminating than the conventional profile. The authors attributed these results to the context and objective of applying each method and also the application of the GPA as a statistical tool for evaluating the results in the conventional profile, because according to them when this treatment is applied in the data of the panel it ends up masking the differences of understanding of the attributes existing between the assessors, which, therefore, leads to a reduction of the discriminating power of

the panel as a whole. In the study conducted by Albert and collaborators (2011), the authors concluded that there was a good correlation between the sensorial maps obtained by the different methodologies studied.

Given the results and discussions of other studies cited above, it was possible to verify that the present study resulted in satisfactory and similar conclusions. The present study revealed little differentiated sensorial profiles among the evaluated products, that is, the new formulation tested showed low differentiation with respect to perceptions and sensorial preferences and also to the survey of attributes by the panel of assessors regarding control samples, which, a priori, the panel has greater sensory contact.

Also, in relation to the results of the profiles, it was obtained a mean of attributes evaluated by the assessors between 8 and 9 attributes, among which were separated into categories of appearance, odor, texture and taste (Figure 1). From the total of 55 attributes surveyed, 15 terms of appearance, 11 of odor, 14 of texture and 15 of flavor were obtained (Table 3). The highest citation attribute was "taste intensity" (8) and twenty-eight attributes were verified with only one citation. Also with regard to the mentioned attributes, there was a great similarity of terms, such as "creaminess" and "creamy", "vinegar odor" and "vinegary odor", and high frequency of citation of the term "intensity". Another observation was referring to the citation of terms, at first sight with opposite meaning, as "salty taste" and "sweet taste". The results were analyzed in the first three dimensions, which were responsible for the explanation of 59.22% of the results (Figure 1 and 2).

Figure 1. Histogram of the attributes number evaluated by assessor

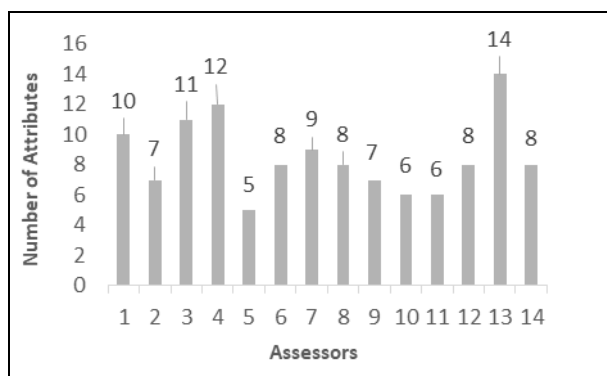


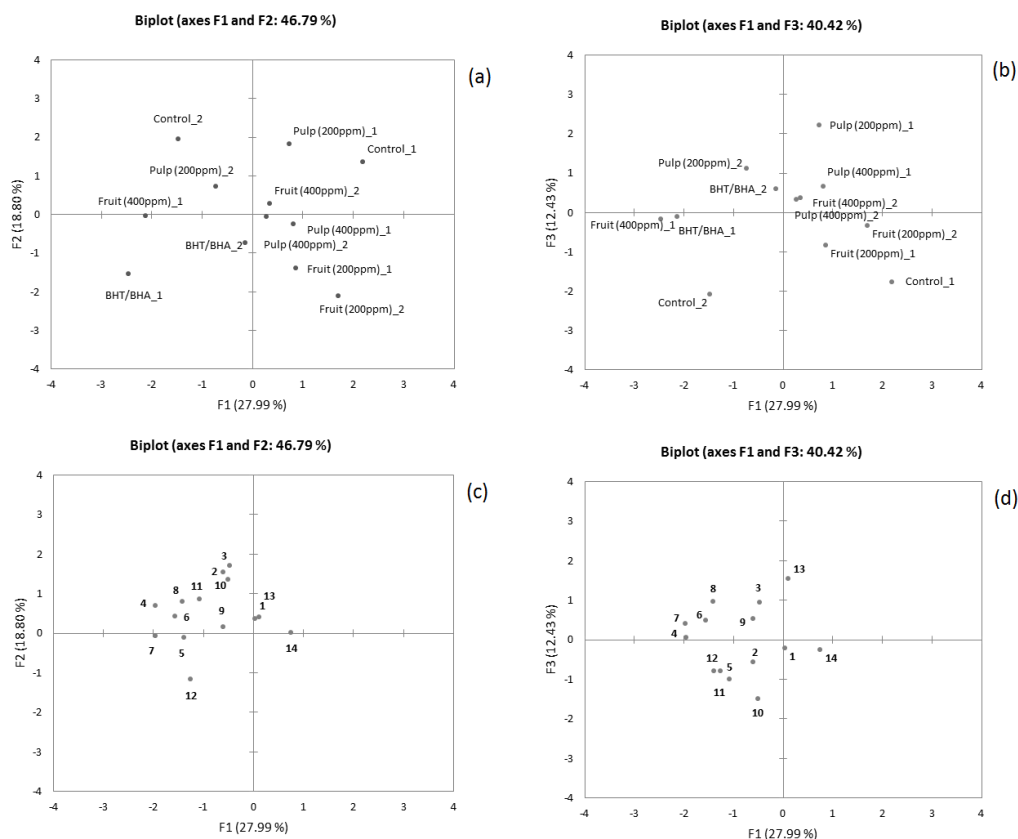
Table 3. Frequency of attributes cited (Appearance, odor, texture and taste)

Attribute	Number of times it was used or cited
<i>APPEARANCE</i>	
Creaminess	3
Creamy	4
Consistency	5
Solid	2
Thickness	1
State/liquid	1
Greasy	1
Presence of bubbles	1
Color	1
Color intensity	2
Yellowish color	1
Cream color	2
Bright color	1
Bright	1
Bright color intensity	1
<i>ODOR</i>	
Spoiled odor	1
Odor intensity	7
Vinegar odor	3
Vinegary odor	3
Vinegary	1
Vinegary odor intensity	2
Vinegar odor intensity	2
Neutral odor	1
Acid odor	1
Acid	1
Olive oil odor	1
<i>TEXTURE</i>	
Creaminess	7
Creamy	4
Consistency	5
Consistency (thick/liquid)	1
Thickness	1
Thick	2
Hardness	1
Dense	1
Soft	3
Soft texture	1
Slim	1
Fresh	5
Smooth	3
Softness	2
<i>TASTE</i>	
Spoiled taste	1
Taste intensity	8
Buttery taste	2
Vinegar taste	3
Vinegary taste	1
Vinegary taste intensity	2
Vinegar taste intensity	1
Acidity intensity	1
Acidity	4
Spice	2
Salty taste	1
Salt quantity	3
Sweet taste	1
Sweetness	1
Greasy	1

For the distribution of samples and assessors in the three dimensions, it can be observed that the control sample showed greater proximity of profile and sensorial description, of the samples added of 200 ppm of acerola pulp and 400 ppm of acerola fruit, being the sample added of the mixture of synthetics antioxidants a more distinct and distant of sensory description from the others. With this, it

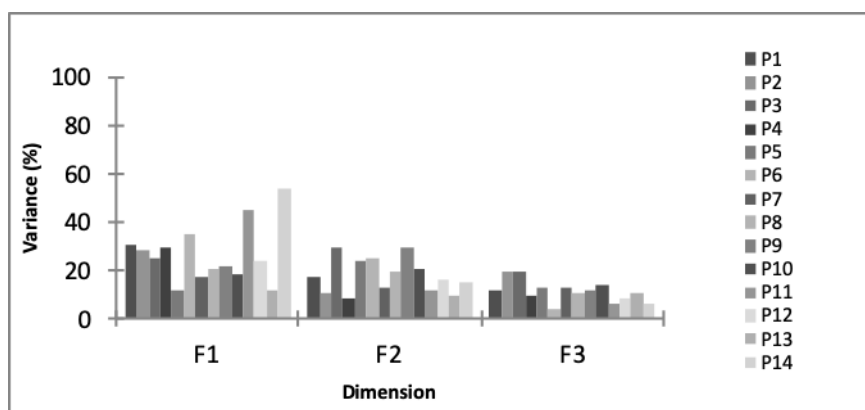
can be verified that the samples, regarding the descriptive profile, did not present sensorial differences for the evaluated attributes. Regarding the distribution of the assessors in the three dimensions, it was verified that they are very close to each other, thus demonstrating that there was a significant consensus regarding the descriptive profile as well the differences and similarities of the samples for the evaluated attributes (Figure 2).

Figure 2. General distribution for the samples in dimensions F1/F2 (a) and F1/F3 (b) and for the assessors in F1/F2 (c) and F1/F3 (d)



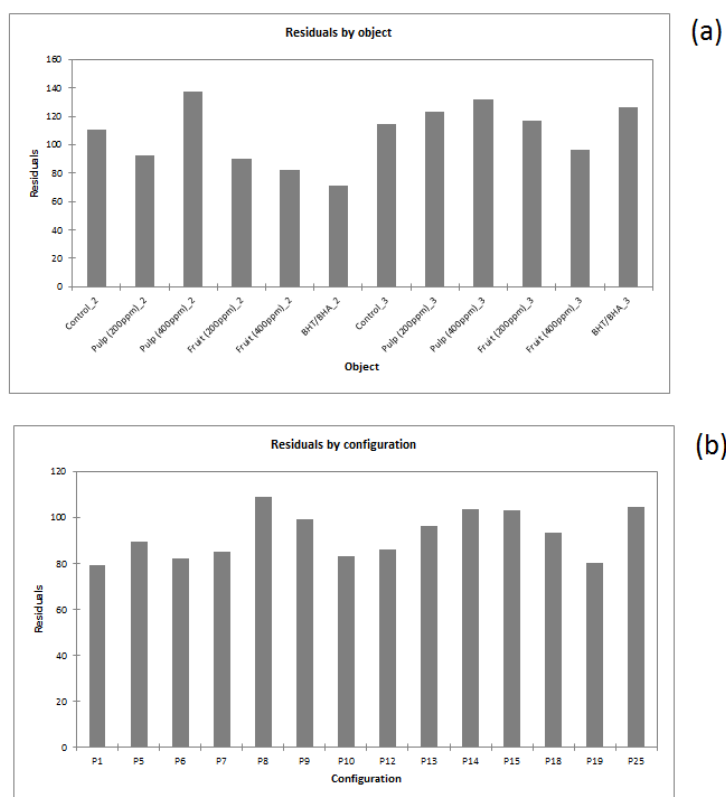
Observing the variance for assessor in the three dimensions, it was concluded that smaller variations occurred in dimensions F2 and F3, ranging from 11.4% to 54.3% in F1, 8.9 to 29.5% in F2 and 3.7 to 20.0% in F3. The lowest variances of each dimension were verified for assessors P13 (11.4%), P4 (8.9%) and P6 (3.7%), respectively (Figure 3). In general, the variance was shown to be low for both assessors and samples, thus reflecting a consensus among the assessors regarding the sensorial scores attributed in the different attributes evaluated for the samples.

Figure 3. Variance distribution (%) for assessors in three dimensions (F1, F2 e F3)



Regarding the residues, responsible for explaining and normalizing the data forms, it was observed that the largest residue obtained was from the assessor P1 (79.3) and the smallest was the assessor P5 (109.1), and ranged on average of 92.4 for the assessors. The residues of the samples ranged from 107.8, with the highest residue of sample added of 400 ppm of acerola pulp (137.0) and the smallest of sample added to the synthetic antioxidant (71.4) (Figure 4).

Figure 4. Residues distribution for samples (a) and assessors (b)



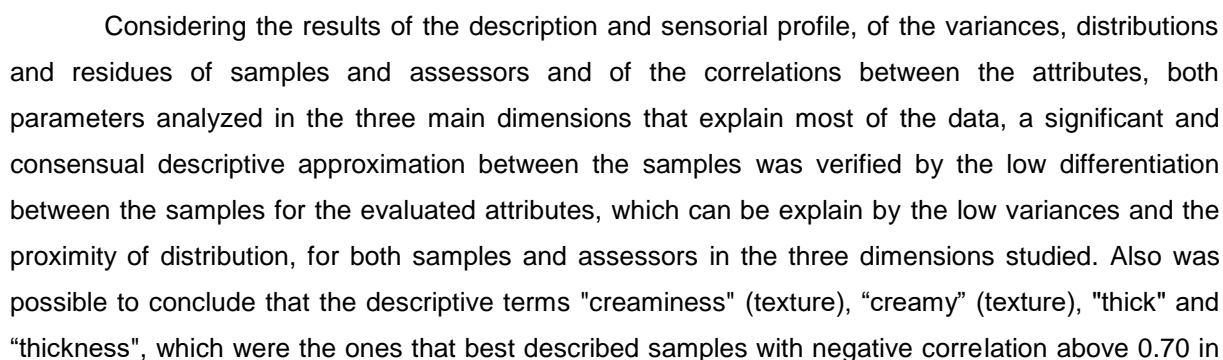
The selection of the most relevant attributes in the sensorial descriptions of the samples was determined by evaluating in addition to the frequency of citation criteria, the observation of variances,

distributions and residues of samples and assessors and the correlation of the terms in the three dimensions (Table 4). Regarding this parameter, the terms that presented correlation greater than or equal to 0.70 were selected as relevant and significant to the descriptive profile. Therefore, the terms "creaminess" (texture), "cream" (texture), "thick" and "thickness" were considered the main descriptors of the samples with negative correlation above 0.70 in the F1 dimension and significant citation frequency. The low correlation obtained for the other terms reinforces the difficulty in describing and applying the ordering of the samples due mainly to the low differentiation between the samples, which can be explained by the lack of training in the most important sensorial attributes to the product and by the diversity/divergence of generated terms.

Table 4. Assessors' attributes correlation in the three dimensions (F1, F2 and F3)

Assessor	F1	F2	F3
1	creaminess (appearance) (-0.70); spoiled odor (0.74); creaminess (texture) (-0.70); dense (texture) (-0.70)	greasy (appearance) (-0.59); odor intensity (0.77); taste intensity (0.14); acid (0.74)	vinegar odor (-0.62); spoiled taste (-0.67)
2	thickness (texture) (-0.84); creamy (texture) (-0.74); taste intensity (-0.46); vinegar taste (0.17)	buttery taste (-0.40)	odor intensity (-0.69); vinegary odor (-0.73);
3	color (0.60); creamy (texture) (-0.68); thick (texture) (-0.72); fresh (texture) (-0.59); soft (texture) (-0.61)	consistency (appearance) (0.79); odor intensity (0.85); vinegar taste (0.59); spice (0.65)	solid (appearance) (0.76); vinegary odor intensity (-0.63)
4	bright (0.53); creamy (appearance) (0.51); consistency (appearance) (-0.62); odor intensity (-0.53); vinegary odor intensity (-0.55); smooth (texture) (-0.62); creamy (texture) (-0.56); fresh (texture) (-0.40); soft (texture) (-0.49); vinegary taste intensity (-0.61)	solid (appearance) (0.29)	acidity intensity (-0.49)
5		Vinegary odor (0.68); taste intensity (0.49)	creamy (appearance) (0.39); soft texture (0.52); consistency (texture) (0.48)
6	consistency (texture) (-0.68); creaminess (texture) (-0.72); taste intensity (-0.78); vinegary taste (-0.49); salty taste (-0.51)	bright color (0.61); odor intensity (0.58); vinegary odor (0.74)	
7	acid odor (0.27); vinegar odor (-0.49); neutral odor (-0.34); soft (texture) (-0.61)	consistency (appearance) (-0.70); smooth (texture) (-0.42)	creamy (appearance) (0.60); creaminess (texture) (0.40); buttery taste (0.57)
8	yellowish color (0.42); cream color (0.42); creamy (appearance) (-0.59); creamy (texture) (-0.75)	vinegary (-0.34); vinegary taste intensity (0.47); acidity (0.53)	fresh (0.61)
9	vinegar odor intensity (-0.83); consistency (texture) (-0.68)	creaminess (texture) (-0.25); taste intensity (-0.64); greasy (taste) (-0.74); salt quantity (-0.59)	color intensity (-0.52)
10	creaminess (texture) (0.52); smooth (texture) (-0.23)	vinegar odor (0.64); vinegar taste intensity (0.45)	bright color intensity (0.49); creaminess (texture) (-0.52); fresh (texture) (-0.51)
11	state/liquid (0.85); presence of bubbles (-0.51); creaminess (texture) (-0.62); consistency (texture) (-0.80)	softness (texture) (0.58);	acidity (-0.44)
12	hardness (texture) (-0.79); softness (texture) (0.32); salt quantity (0.69)	creaminess (appearance) (-0.62); odor intensity (0.48)	acid (odor) (-0.44); acidity (0.20); taste intensity (0.41)
13	olive oil odor (0.42); consistency (thick/liquid) (texture) (-0.61); sweet taste (-0.49); spice (0.34)	consistency (appearance) (0.33); creaminess (appearance) (-0.31); odor intensity (-0.64); sweetness (-0.42)	color intensity (-0.46); cream color (0.36); consistency (texture) (0.28); fresh (texture) (0.69); taste intensity (-0.31); salt quantity (0.57)
14	thickness (appearance) (-0.88); consistency (appearance) (-0.88); creaminess (texture) (-0.74); slim (texture) (0.89); thick (texture) (-0.89)	taste intensity (0.53); vinegar taste (0.49)	vinegar odor intensity (-0.36)

When analyzing the distribution of the attributes in the dimensions, it was observed that the terms "consistency", "creaminess" and "creamy" are distributed very closely, thus demonstrating a consensus among the assessors regarding these attributes. Another relevant observation is related to the terms and attributes that present the word "vinegar" or "vinegary" ("vinegar odor", "vinegary odor", "vinegar taste", "vinegary taste"), these terms were closely distributed among each other. Attributes related to color and taste also showed some closeness. However, when assessing the distribution of the attributes in a global way, there is a greater consensus on the appearance and texture attributes



the F1 dimension and significant citation frequency, however the low correlation obtained for other terms reinforces the consensual difficulty in describing and apply the ordering of the samples, due to the small differences between the samples.

As a general conclusion, when comparing the results of the present descriptive study with other studies, it has been that the more modern descriptive methods like flash profile (FP) can be used as fast alternatives to the classical method (QDA) and in research studies with consumers because they do not demand for trained panels of assessors. Flash Profile, as a general conclusion, can be used as a preliminary sensory mapping tool in contexts of broader or complete descriptive sensory studies, in mapping studies of consumer preferences, and may even be used to assist the development stage of language and terms/attributes of a conventional profile. In addition, it can be applied in the sensory evaluation of shorter *shelf life* products or in situations where there is no possibility to perform more than one sensory test session in the study, that is, whenever it is necessary to obtain a rapid sensory positioning of a group of products (Delarue & Sieffermann, 2004, Moniez, Truchot & Sieffermann, 2001). As a general rule, Flash Profile can be used satisfactorily in three main sensory situations: when it is desired to obtain rapid sensory responses (profiles and descriptions), as an initial screening tool for sensory perception of a new product or a new category of products, and to study specific markets (Dairou & Sieffermann, 2002; Delarue & Sieffermann, 2004; Tarea, Cuvelier & Sieffermann, 2007; Varela & Ares, 2012

3.2 Global Acceptance test

The results obtained in the acceptance analysis reflected and agreed with the results of the sensorial profile of the samples. In the sensory profile, a high consensus was found, low discriminative power between the assessors was verified and confirmed by the low variances of both the assessors and the samples, due mainly to the low differences between the samples (low differentiation between the formulations), thus demonstrating that the substitution of synthetic antioxidants by the acerola pulp or whole fruit does not impair the organoleptic and sensorial quality of the product, being slightly perceptible and of significant sensorial acceptance. In addition to the results obtained, it is possible to observe a consensus among the assessors in the survey and description of attributes (profile) and in ordering the samples in relation to the selected attributes.

Table 5. Grades of acceptance for mayonnaises

Sample	Acceptance Grades
Control	6.9±1.5 b
Acerola Pulp (200 ppm)	7.0±1.7 b
Acerola Pulp (400 ppm)	7.2±1.3 b
Acerola Fruit (200 ppm)	6.9±1.5 b
Acerola Fruit (400 ppm)	7.6±1.1 a
BHA+BHT (100+100 ppm)	7.1±1.5 b

Mean of 60 assessors ± standard deviation (SD)

Same letters in column not differ significantly for $p \leq 0.05$ ($\alpha = 5\%$)

Regarding the acceptance, the sample with the highest concentration of acerola fruit (400 ppm) was the one that obtained the highest sensory rating and greater acceptance, being statistically different from the others. The other samples did not obtain significant differences between them about their notes of sensory acceptance. Similar sensorial acceptance was observed among the control samples with the samples added of low concentrations of natural antioxidants (200 ppm of acerola pulp and 200 ppm of acerola fruit), which were the lowest acceptance test samples, 6.9 for the control and for the sample added of 200 ppm of acerola fruit and 7.0 for sample added of 200 ppm of acerola pulp. Samples with the highest concentration of natural antioxidants (400 ppm of acerola pulp and 400 ppm of acerola fruit) also presented similar sensory acceptance scores, however, these obtained the highest acceptance scores, 7.6 for the sample added of 400 ppm of acerola fruit and 7.2 for the sample added of 400 ppm of acerola pulp (Table 5 and 6). The sample added of the synthetic antioxidant blend presented a median sensory score of 7.1.

Table 6. Samples correlation (p-value) by Wilcoxon test

	Control	Acerola Pulp (200 ppm)	Acerola Pulp (400 ppm)	Acerola Fruit (200 ppm)	Acerola Fruit (400 ppm)	BHA+BHT (100+100 ppm)
Control		0.836	0.111	0.879	0.002*	0.408
Acerola Pulp (200 ppm)	0.836		0.286	0.823	0.020*	0.816
Acerola Pulp (400 ppm)	0.111	0.286		0.107	0.032*	0.445
Acerola Fruit (200 ppm)	0.879	0.823	0.107		0.002*	0.365
Acerola Fruit (400 ppm)	0.002*	0.020*	0.032*	0.002*		0.023*
BHA+BHT (100+100 ppm)	0.408	0.816	0.445	0.365	0.023*	

P-Value with confidence interval of 95% ($p \leq 0.05$)

* Samples with no significant correlation between them ($\alpha = 5\%$)

Given that the sensorial acceptance notes were very close, with only sample added of 400 ppm of acerola fruit statistically different from the others with the highest grade, it is concluded that the substitution of synthetic antioxidants by antioxidants from natural sources such as acerola in emulsions is satisfactory and does not promote significant sensorial differences of acceptance and can be performed without impairment of the sensory and sensorial quality.

4. Conclusions and Final Considerations

Both descriptive (qualitative) and hedonic/affective (quantitative) sensory evaluation methods are of great importance in relation to the direction of the industry in the area of product development, and they are complementary and auxiliary, as allow the development and construction of identity and quality parameters of food products. By aligning and correlating the results and information of these two techniques, it is possible to describe and position a product within a class of products, and also to size the sensorial impact, more linked to affectivity, that new products may have on consumers. For mayonnaise added of acerola antioxidants the sensorial evaluation showed a high consensus of the assessors in the description of attributes as well as in the ordering of the same for the sensorial attributes listed as best descriptors of the product. Even with a high consensus, it was also possible to verify the low discriminant power of the assessors, confirmed by the low variances and proximity of both assessors and samples, because the samples presented few differences in formulation (only the antioxidant, as well as concentration were different). Reinforcing and complementing the results and conclusions obtained with the profile, there was still a relative proximity of sensorial acceptance notes, being only one sample different from the others (added with 400 ppm of acerola).

The combination of the two techniques allowed to conclude that the substitution of synthetic antioxidants by the pulp or fruit of acerola does not cause damages to the sensorial quality of the product, being little perceptible and of significant sensorial acceptance.

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GENERAL CONCLUSION

This work revealed that the acerola presents a potential application as an antioxidant source for lipid base emulsion, representing a satisfactory alternative to the synthetic ones, due to its high content in bioactive compounds. It was possible to identify the ideal extraction condition to recover antioxidant compounds from whole acerola fruits, seeds and pulp. Finally, the results of the sensory evaluation study allowed to conclude that acerola, as an ingredient and source of antioxidants, has great potential for application in the food industry.