UNIVERSIDADE ESTADUAL DE CAMPINAS
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RICKYN ALEXANDER JACINTO VALDERRAMA

EFFECT OF THE APPLICATION OF RASPBERRY (*Rubus idaeus*) AND BLACKBERRY (*Rubus brasiliensis*) PULP PROCESSING BY-PRODUCTS ON PHYSICOCHEMICAL CHARACTERISTICS, ANTIOXIDANT CAPACITY, OXIDATIVE AND SENSORY STABILITY OF CHICKEN BURGER

EFEITO DA APLICAÇÃO DE SUBPRODUTOS DO PROCESSAMENTO DE POLPA DE FRAMBOESA (*Rubus idaeus*) E AMORA-PRETA (*Rubus brasiliensis*) NAS CARACTERÍSTICAS FÍSICO-QUÍMICAS, CAPACIDADE ANTIOXIDANTE, ESTABILIDADE OXIDATIVA E SENSORIAL DE HAMBÚRGUER DE FRANGO

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Orientadora: Prof[a]. Dr[a]. Priscilla Efraim

Co-orientadora: Dr[a]. Kazumi Kawasaki Ramos

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Aos meus pais Felipe e Martha,
e a Camila, minha esposa
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“Nos melhores livros buscai palavras de sabedoria; procurai conhecimento, sim, pelo estudo e também pela fé”

(Jesus Cristo)

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RESUMO

O Brasil é o terceiro maior produtor de frutas do mundo, e estima-se que, em média, 45% da produção mundial de frutas e hortaliças é descartada nos diferentes processos produtivos. Os subprodutos do processamento de frutas contêm compostos bioativos benéficos para a saúde humana. O hambúrguer é um dos produtos cárneos de maior consumo no mundo, porém, o tipo de carne, as condições de processamento, cozimento e armazenamento aceleram as reações oxidativas na matriz cárnea, afetando as características sensoriais e a vida útil do produto. Dessa forma, se faz necessário o uso de antioxidantes para inibir ou retardar a oxidação lipídica nesse tipo de produto. Os subprodutos do processamento de frutas vermelhas apresentam compostos fenólicos com propriedades antioxidantes, e a sua aplicação na produção de hambúrguer de frango pode auxiliar na obtenção de um produto mais saudável. Neste sentido, o presente trabalho teve como objetivo caracterizar os subprodutos do processamento de polpa de framboesa (*Rubus idaeus*) e amora-preta (*Rubus brasiliensis*), e avaliar o efeito da sua aplicação nas características físico-químicas, estabilidade oxidativa e características sensoriais de hambúrguer de frango. O trabalho foi conduzido em três etapas: (1) Caracterização dos subprodutos do processamento de polpa de framboesa e amora-preta (SF e SA, respectivamente) quanto às propriedades físico-químicas; (2) Adição de 2% de SF e SA em formulação padrão de hambúrguer de frango, em hambúrguer com 73% de carne de frango (FA) e em hambúrguer com 53% de carne de frango e 20% de CFMS (FB). O hambúrguer FA cru foi armazenado a -18 ºC durante 60 dias, e o hambúrguer FB foi cozido e armazenado em BOD a 4 ºC por 5 dias; (3) Caracterização dos hambúrgueres quanto às propriedades físico-químicas, capacidade antioxidante, perfil de textura, características de cozimento e oxidação lipídica. Testes sensoriais foram realizados (aceitação e CATA). Os SF e SA apresentaram valores de 26,76 mg e 33,05 mg GAE/g de sbs para compostos fenólicos totais; 699,33 µmol e 781,49 µmol equivalentes de trolox/g de sbs para a capacidade antioxidante; e 54,41 g e 55,85 g de fibras totais/g de sbs, respectivamente. Em média, a adição de SF e SA em hambúrguer de frango aumentou o conteúdo de lipídios em 25,95%, de compostos fenólicos totais em 122,45%, e a capacidade antioxidante em 23,24%. Não houve diferença entre os tratamentos quanto às características de cozimento, porém, no perfil de textura, houve redução da dureza, elasticidade, coesividade e mastigabilidade ao longo do tempo de armazenamento para os tratamentos com adição de SF e

SA. A adição de SF e SA em hambúrguer de frango inibiu a oxidação lipídica durante o tempo de armazenamento, chegando a superar o efeito antioxidante do ascorbato de sódio. A aceitação sensorial das amostras com SF e SA foi moderada, sugerindo a necessidade de ajustes na formulação. Os atributos de maior frequência no CATA para o tratamento com adição de SF foram: seco, textura firme e sabor de fruta, e para o tratamento com adição de SA foram: gosto amargo, sabor residual e aparência de hambúrguer bovino.

Palavras-chave: Subprodutos, compostos bioativos, hambúrguer de frango, oxidação lipídica.

ABSTRACT

Brazil is the third largest producer of fruit in the world, and it is estimated that, on average, 45% of world fruit and vegetable production is wasted in the different production processes. By-products of fruit processing contain bioactive compounds beneficial to human health. Burger is one of the most consumed meat products in the world, however, the type of meat, processing, cooking and storage conditions accelerate the oxidative reactions in the meat matrix, affecting the sensory characteristics and the useful life of the product. However, it is necessary to use antioxidant to inhibit or retard lipid oxidation in this type of meat. By-products of the processing of berries have phenolic compounds with antioxidant properties, and their addition in the production of chicken burger can help in obtaining healthier product. In this sense, the present work aimed to characterize the by-products of the processing of raspberry (*Rubus idaeus*) and blackberry (*Rubus brasiliensis*) pulp and to evaluate the effect of its application on physicochemical, oxidative stability and sensorial characteristics of chicken burger. The work was conducted in three stages. (1) Characterization of the by-products of the raspberry and blackberry pulp processing (RB and BB, respectively), regarding the physicochemical properties; (2) Addition of 2% of RB and BB in standard chicken burger formulation, in burger with 73% of chicken meat (FA), and 53% of chicken meat and 20% of MSCM (FB). The raw FA burger was stored at -18 °C for 60 days, and the FB burger was cooked and stored in BOD at 4 °C for 5 days. (3) Characterization of the chicken burgers on the physicochemical properties, antioxidant capacity, texture profile, cooking characteristics and lipid oxidation. Sensory tests were performed (acceptance and CATA). RB and BB presented values of 26.76 mg and 33.05 mg GAE/g bdb for total phenolic compounds; 699.33 μmol and 781.49 μmol trolox equivalents/g bdb for antioxidant capacity; and 54.41 g and 55.85 g of total fibers/g bdb, respectively. On average, the addition of RB and BB in chicken burger increased the lipid content by 25.95%, total phenolic compounds by 122.45%, and antioxidant capacity by 23.24%. There was no difference between the treatments in the cooking characteristics, however, in the texture profile; there was a reduction in hardness, elasticity, cohesiveness and chewing throughout the storage time for the treatments with addition of RB and BB. The addition of RB and BB in chicken burger inhibited lipid oxidation during storage time, even exceeding the antioxidant effect of sodium ascorbate. The sensorial acceptance of the RB and BB samples was moderate, suggesting the need to adjust in the formulation. The attributes of higher frequency in

the CATA for the treatment with addition of RB were dry, firm texture and fruit flavor, and for the treatment with addition of BB were bitter taste, residual taste and appearance of beef burger.

Keywords: By-products, bioactive compounds, chicken burger, lipid oxidation.

SUMÁRIO

INTRODUÇÃO GERAL

O Brasil é o terceiro maior produtor de frutas do mundo, com produção estimada em cerca de 40 milhões de toneladas de frutas frescas ao ano, e perspectiva de se tornar o maior fornecedor de alimentos do mundo na próxima década (FAO[a], 2015). A demanda por alimentos continua em aumento, seguindo o crescimento populacional do mundo, que se estima será de 8,6 bilhões de pessoas para o 2030, com um aumento de 83 milhões de pessoas ao ano (FAO, 2017). Estima-se que das frutas frescas produzidas no Brasil, 47% é destinada para o processamento de produtos como polpas, sucos e néctares, das quais, o 26% é exportado (SEBRAE, 2016).

A perda e o desperdício são alguns dos maiores problemas observados na cadeia de produção e processamento de alimentos, e estima-se que, em média, 45% da produção mundial de frutas e hortaliças são descartados nos diferentes processos produtivos (FAO[b], 2015). A elevada perda e o grande desperdício da produção de frutas provocam prejuízos econômicos, ambientais e diminuição na segurança alimentar (Agovino, Cerciello, & Gatto, 2018).

Durante o processamento de frutas, são descartados subprodutos tais como cascas, sementes e partes pouco apreciadas da fruta, que representam entre 20-30% do peso da fruta inteira (Salaheen, Peng, & Biswas, 2015). Os subprodutos de frutas vermelhas, como a amora-preta e a framboesa são ricos em nutrientes, fibras dietéticas, corantes e compostos fenólicos (Kao, & Chen, 2013; Salaheen, Peng, & Biswas, 2015). Estudos sugerem que o seu consumo reduz o risco de desenvolvimento de doenças como o câncer, hipertensão, inflamação e problemas cardiovasculares entre outros (Bowen-Forbes, Zhang, & Nair, 2010; Liobikas, Skemiene, Trumbeckaite, & Borutaite, 2016). Por essa razão, tem aumentado o interesse em aproveitar os subprodutos de frutas e seus derivados como ingrediente alimentar, com a finalidade de reduzir e/ou substituir o uso de aditivos na elaboração de alimentos (Kadagoda & Marapana, 2017).

A carne de frango é uma das carnes com maior aceitação no mundo, e possui em sua composição, maior teor de fosfolipídios em comparação com outras carnes, e que por sua vez, estão constituídos, em maior proporção, por ácidos graxos poli-insaturados (Wilson, Pearson & Shorland, 1976). A presença dos ácidos graxos poli-insaturados faz com que a carne de frango seja mais susceptível à oxidação lipídica (Dawson, & Spinelli, 2012). A carne de frango mecanicamente separada (CFMS) é obtida do esmagamento dos tecidos após a remoção da carne da carcaça de frango e também é altamente susceptível à oxidação lipídica (Negrão et al., 2005).

O hambúrguer é um dos produtos cárneos de maior consumo no mundo, com uma ampla variedade de formulações dependendo do tipo de carne, custos de produção e valor nutricional (Feiner, 2006). As condições de processamento do hambúrguer tais como a moagem e adição de sal, a taxa de congelamento, condições e tempo de armazenamento aceleram as reações de oxidação durante o cozimento (Gheisari, & Motamedi, 2010; Soyer, Özalp, Dalmış, & Bilgin, 2010). Em pouco tempo desenvolvem sabores e aromas ranços, formados a partir da transformação dos ácidos graxos em compostos de cadeia curta, tais como aldeídos, cetonas, álcoois e ácidos (Rhee, Anderson, & Sams, 2005).

A utilização de antioxidantes sintéticos retarda ou inibe reações oxidativas. Porém, há indícios de que esses aditivos alimentares podem prejudicar a saúde em função de efeitos toxicológicos (Sarafian, Kouyoumjian, Tashkin, & Roth, 2002). A aplicação de subprodutos de frutas vermelhas na reformulação de hambúrguer de frango é uma alternativa que pode suprir a necessidade de um melhor aproveitamento dos subprodutos do processamento de frutas, e que pode auxiliar na obtenção de hambúrgueres de frango mais saudáveis ou chamados de "*Clean Label"* e, que ao mesmo tempo, propiciem propriedades funcionais e nutracêuticas ao alimento (Kowalska, Czajkowska, Cichowska, & Lenart, 2017; Liobikas et al., 2016).

Este trabalho foi divido em três capítulos. O Capítulo 1 apresenta uma revisão bibliográfica sobre a importância da utilização dos subprodutos do processamento de frutas na reformulação de produtos cárneos, as aplicações, desafios e alternativas. O Capítulo 2 descreve a caracterização do subproduto do processamento de polpa de framboesa e amora-preta, e o efeito da sua aplicação nas características físico-químicas e propriedades funcionais em hambúrguer de frango. Por fim, o Capítulo 3 descreve o efeito da aplicação de subproduto do processamento de polpa de framboesa e amora-preta na oxidação lipídica e nas características sensoriais de hambúrguer de frango.

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OBJETIVO

OBJETIVO GERAL

Avaliar o efeito da aplicação de subprodutos do processamento de polpa de framboesa (*Rubus idaeus*) e amora-preta (*Rubus brasiliensis*) nas características físico-químicas e sensoriais de hambúrguer de frango.

OBJETIVOS ESPECÍFICOS

a. Caracterizar os subprodutos de framboesa (*Rubus idaeus*) e amora-preta (*Rubus brasiliensis*) quanto às características físico-químicas.

b. Avaliar o efeito da aplicação de subprodutos de framboesa (*Rubus idaeus*) e amora-preta (*Rubus brasiliensis*) nas características físico-químicas em hambúrguer de frango.

c. Avaliar o efeito da aplicação de subprodutos de framboesa (*Rubus idaeus*) e amora-preta (*Rubus brasiliensis*) na estabilidade oxidativa em hambúrguer de frango ao longo do tempo de armazenamento.

d. Avaliar o efeito da aplicação de subprodutos de framboesa (*Rubus idaeus*) e amora-preta (*Rubus brasiliensis*) na estabilidade sensorial em hambúrguer de frango ao longo do tempo de armazenamento.

CHAPTER 1

Review: Fruit processing by-products in the reformulation of meat products: applications, challenges and alternatives

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This chapter will be submitted to the *Trends in Food Science & Technology* journal

Review: Fruit processing by-products in the reformulation of meat products: applications, challenges and alternatives

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ABSTRACT

Background: the bioactive compounds present in fruit processing by-products are an alternative in the reformulation of meat products to obtain "Clean Label" and healthier foods.

Scope and approach: the objective of this review is to point out the opportunities of applying by-products generated and discarded by the fruit processing industry to obtain "healthier" meat products. The most important bioactive compounds present in fruit by-products will be highlighted, as well as the alternatives of its application in the reformulation of meat products, aiming to replace food additives for healthier ingredients. Challenges in trying to combine fruit by-products with the meat matrix will also be approached, and some alternatives to improve sensory quality.

Key findings and conclusion: researches suggest that fruit processing by-products are rich in dyes, dietary fibers, antioxidants, antimicrobials and other bioactive compounds, and that its use is viable in the processing of reformulated meat products and, in this way, to obtain stable and safe food, as well as, rich in bioactive compounds. Thus, the use of fruit processing by-products is an alternative to improve agro-industry sustainability and to overcome the challenges of the meat industry.

Keywords: By-products, reformulation, clean label, meat product, bioactive compounds.

1. Introduction

The biggest problems in food production are losses and waste, of which an average of 45% of world fruit and vegetable production is lost in the field, during the post-harvest handling,

processing, distribution, storage, retail and bad habits of purchase by the final consumer, according to the report of the Food and Agriculture Organization of the United Nations (2015). Losses and waste bring economic, environmental and against food security problems. This situation make agricultural activity unsustainable in our current reality, where the demand for food continues to increase, food price volatility disadvantages the most needed, and agricultural production is responsible for 10 to 15% of the planet's greenhouse effect gas emissions, contributing significantly to climate change (Agovino, Cerciello, & Gatto, 2018).

During the processing of fruits, waste originates when is used the part of the fruit considered most valuable, leaving by-products such as peels, seeds and other less appreciated parts. Nevertheless, in recent years, the interest in making better use of agricultural by-products for different purposes has increased, including its application as reinforcement of polymer composites (Binoj, Raj, & Indran, 2018), to obtain biohydrogen (Keskin et al., 2018), as adsorbents of contaminating compounds (Dai et al., 2018), to obtain compounds and food additive (Kadagoda & Marapana, 2017), among other applications.

Nutrients such as proteins, lipids, dietary fibers, vitamins, minerals, as well as color and antioxidant and antimicrobial compounds, are eliminated along with fruit waste for not complying with standards of quality and acceptance as part of the perception, belief and consumption habits of the population (Nikolaus, Nickols-Richardson, & Ellison, 2018). However, many fruit by-products can be used by the food industry itself; their use can bring economic, environmental and social benefits, making fruit processing more sustainable (Ribeiro, Sobral, & Henriques, 2018).

There is a growing demand for "healthier" and "clean label" foods that help with the reduction or substitution of food additives by healthier natural sources (Gouw, Jung, & Zhao, 2017). Besides, the International Agency for Research on Cancer (IARC), which is part of the World Health Organization of the United Nations, classified meat products as carcinogenic to

humans (WHO, 2015). Therefore, the application of fruit processing by-products in the reformulation of meat products is an alternative that can supply the need to better use of generated waste by the processed fruits industry, and to obtain bioactive ingredients of utility to meat industry (Kowalska, Czajkowska, Cichowska & Lenart, 2017).

In this context, the objective of this review manuscript is to highlight the agro-industrial potential and the challenges of using fruit processing by-products to obtain healthier reformulated meat products.

2. Bioactive properties of fruit by-products in food industry

Fruit by-products are solid or semi-solid products, obtained after separation of the juice or oily portion, consisting in peels, seeds and small quantities of fruits that represent between 20-30% of the weight of the whole fruit (Salaheen, Peng, & Biswas, 2015). These fruit by-products have a high nutritional and dietary value, and a source of dyes, polysaccharides, and essential oils, among other bioactive compounds (Salaheen, Nguyen, Hewes, & Biswas, 2014). If reincorporated in human nutrition, they can bring important benefits for health, since they have antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antihypertensive and cardioprotective properties (Bowen-Forbes, Zhang, & Nair, 2010; Liobikas, Skemiene, Trumbeckaite, & Borutaite, 2016).

Food security is one of the biggest concerns of the Food and Agriculture Organization (FAO, 2015). Government policies are being developed to decrease food loss and waste. Nutrients such as carbohydrates, lipids, proteins, dietary fibers, vitamins and minerals are daily discarded due to inappropriate administration in the chain of production, distribution, storage and because of the final consumer (Spiker, Hiza, Siddiqi & Neff, 2017). Food technology can help to fight it, using food conservation and processing technologies to improve and maximize productive processes after

harvest, as well as, developing by-product use options, with the aim of eliminating or reducing waste in fruit processing (Bourne, 2017).

Studies suggest that the consumption of dietary fibers brings benefits to health: decreases the risk against colon cancer (Chen, & Vietta, 2018) and cardiovascular diseases (Crowe et al., 2012), stimulates the intestinal transit (Brodribb, Condon, Cowles, & DeCosse, 1980), influences on satiety (Golay, & Bobbioni, 1997) and helps in the absorption of minerals (Trinidad, Wolever, & Thompson, 1996). In addition, fruit processing by-products, such as bananas, grapefruit, pomegranate, passion fruit, mango peels, watermelon rinds, and kiwi, apple, peach and physalis pomace, have technological potential to extract pectin for use in food (Abid et al., 2017; Mandalari et al., 2006).

The use of fruit processing by-products can contribute to the reduction of food insecurity. In addition, the addition of dietary fibers in food reformulation may benefits the consumer's health. Table 1 shows the centesimal composition and content of dietary fiber in some fruit processing by-products.

Color is one of the attributes that influences directly on consumer preference, and is related to food quality. Dyes are widely used in the food industry to improve the attractiveness of the products (Basanta et al., 2018). Synthetic dyes feature good stability to light, heat and pH, however, adverse reactions related to their consumption, including allergic reactions and attention deficit hyperactivity disorder (Bhatt et al., 2018; Feketea, & Tsabouri, 2017). That is why healthier dyes have gained more attention from consumers (Román, Sánchez-Siles, & Siegrist, 2017). Natural dyes are obtained from fruits and vegetables, such as chlorophyll, carotenoids, betalains, flavonoids and anthocyanins. Fruit processing by-products are a source of natural dyes, which can help in obtaining food preparation by reducing or replacing dyes that are harmful to health (Kao, & Chen, 2013), as described in Table 2.

Table 1. Centesimal composition, insoluble and soluble total dietary fibers of fruit processing by-products

Fruit	By-product	Protein (%)	Lipids (%)	Ashes (%)	Carbohydrates (%)	D.F. Total (%)	D. F. Insoluble (%)	D. F. Soluble (%)	Source
Gooseberry	Peel and seed	11.1-13.3**	5.9-10.8**	2.8-3.3**	69.8-71.9**	72.0-77.4*	46.9-47.4*	25.1-30.0*	Alba et al. (2018)
Jabuticaba	Peel and seed	1.26**	0.63**	2.38**	84.23**	20.54**	16.42**	4.12**	Gurak et al. (2014)
Apple	Peel and seed	2.06*	2.7*	0.50*	-	51.10**	36.50**	14.60**	Sudha et al. (2007)
Grape	Peel and seed	10.25*	15.28*	6.08*	1.18*	60.5*	49.3*	11.25*	Nayak et al. (2018)
Passion fruit	Seed and fiber	1.49*	29.54*	5.77*	-	53.51*	48.25*	5.26*	López-Vargas et al. (2014)
Passion fruit	Albedo	0.35*	1.00*	8.08*	-	71.79*	52.34*	19.45*	López-Vargas et al. (2014)
Kiwi	Peel	3.84-4.22	0.75-2.10	4.02-4.16	52.16-53.73	25.85-28.18	18.71-18,92	6.93-9.47	Soquetta et al. (2016)
Kiwi	Seed and fiber	6.68-8.31	12.26-26.10	2.56-3.52	34.53-41.31	28.0-29.44	23.93-26.23	1.77-5.50	Soquetta et al. (2016)
Buriti	Peel	2.59**	0.52**	1.02**	95.87**	88.69*	87.76*	0.94*	Resende et al. (2019)
Melon	Peel	7.48*	2.12*	3.67*	69.77*	41.69*	37.58*	4.38*	Mallek-Ayadi et al. (2017)
Pineapple	Peel and fiber	4.71*	0.61*	2.24*	43.46*	45.22*	44.44*	0.78*	Selani et al. (2014)
Pomelo	Pomace	4.46-8.42*	1.04-3.24*	3.22-3.27*	18.3-45.0*	44.2-62.6*	37.8-56.0*	4.57-6.43*	Figuerola et al. (2005)
Lime	Pomace	6.79-7.92*	1.88*	3.47-3.91*	14.6-25.9*	60.1-68.3*	50.9-62.0*	6.25-9.20*	Figuerola et al. (2005)
Orange	Pomace	6.70*	0.89*	2.71*	17.9*	64.3*	54.0*	10.28*	Figuerola et al. (2005)

- D. F., dietary fiber; * percentage on dry basis; ** percentage on wet basis.

Table 2. Color compounds in fruit processing by-products

Dyes	By-product	Source
Carotenoids	Tomato pomace	Knoblich, Anderson, & Latshaw (2005)
	Pupunha peel	Matos et al. (2019)
	Tucumã peel	Matos et al. (2019)
	Mango peel	Garcia-Mendoza et al. (2015)
	Melon rind	Benmeziane et al. (2018)
Flavonoids	Pomelo	Tian et al. (2018)
	Lime peel	Chen et al. (2019)
	Orange peel	Mandalari et al. (2006)
	Tangerine peel	Min-Jung et al. (2016)
Anthocyanins	Aronia peel	Vagari, & Jensen (2017)
	Plum peel	Hernández-Herrero, & Frutos (2014)
	Blackberry pomace	Vargas et al. (2017)
	Blueberry pomace	Bo et al. (2016)
	Grape wine lees	Romero-Díez et al. (2019)
Betalains	Pitaya peel	Mello et al. (2015)
	Indian fig peel	Soto-Castro et al. (2019)
Lycopene	Seedless tomato pomace	Poojari, & Passamonti (2015)
	Gac arils	Nhung et al. (2010)
	Rindless watermelon pomace	Oberoi, & Sogi (2017)

The food industry uses preservatives as food additives to delay or inhibit oxidative reactions and microbial growth that decreases the shelf life of food. However, studies conclude that there is enough evidence to link the intake of synthetic preservatives in food with endocrine disrupting and carcinogenic potential (Maher et al., 2018). A great variety of bioactive compounds, compounds with antioxidant and antimicrobial activity are present in fruit by-products, such as phenolic acids, catechins, flavonols, flavones, flavanones, anthocyanins, carotenoids, tocopherols, among others (Kao, & Chen, 2013; Salaheen, Peng, & Biswas, 2015). The use of these compounds in our daily diet helps to prevent cardiovascular diseases and cancer (Liobikas et al., 2016). Tables 3 and 4 describe the content of phenolic and anthocyanins in some fruit by-products.

Table 3. Phenolic content in fruit by-products

Fruit	By-product	Phenolic content	Sample weight	Extraction solvent	Source
Grape	Pomace	1949 mg GAE/g dry extract	10 g powder extract	Ethanol 70 %	Zhu et al. (2019)
Pomegranate	Peel	442.48 mg GAE/g dry extract	5 g sample	Methanol	Kharchoufi et al. (2018)
Raspberry	Pomace	26.3-43.7 mg GAE/g dry extract	70 g sample	Methanol 80 % (0.05 acetic acid)	Cetojevic-Simin et al. (2015)
Blackberry	Pomace	75.50-88.28 mg GAE/g dry extract	-	Methanol 80 % (0.05 acetic acid)	Cetojevic-Simin et al. (2017)
Melon	Peel	3.32 mg GAE/100g extract	-	Ethanol 95 %	Mallek-Ayadi et al. (2017)
Apple	Pomace	6.05-8.87 mg GAE/g extract	40 g sample	Supercritical fluid: CO_2 and ethanol	Ferrentino et al. (2018)
Buriti	Peel	7.85-9.35 mg GAE/g dry matter	1,0 g sample	Methanol 50% and Acetone 70%	Resende et al. (2019)
Pomelo	Albedo	12.67-13.37 mg GAE/g dry matter	0.5 g sample	Methanol 80 %	Rahman et al. (2018)
Pomelo	Flavedo	10.45-12.26 mg GAE/g dry matter	0.5 g sample	Methanol 80 %	Rahman et al. (2018)
Orange	Peel	3.61 mg GAE/ g peel	0.5 g sample	Ethylene	Ozturka et al. (2018)
Pitaya	Peel	40.68 mg GAE/100g fresh weight	5,0 g sample	Acetone 80 %	Mello et al. (2015)

* GAE, gallic acid equivalent.

Table 4. Anthocyanins content in fruit by-products

Fruit	By-product	Anthocyanins content	Sample weight	Extraction solvent	Source
Apple	Pomace	0.4-0.78 mg Cya/g fresh weight	1,0 g sample	Methanol 95 % (hydrochloric acid)	Shafiq, & Singh (2018)
Black aronia	Peel	3.84 mg Cya/g fresh weight	2.5 g sample	Methanol 99% (hydrochloric acid)	Vagari, & Jensen (2017)
Jaboticaba	Pomace	5.73 mg Cya/g dry weight	0,1 g sample	Methanol 80%, Acetone 70%	Gurak et al. (2014)
Grape	Pomace	1.72-6.57 mg malvidin/g pomace	-	Ultrasound: ethanol 50%	Carmora-Jiménez et al. (2018)
Gooseberry	Peel	6.43 mg Cya/g peel	-	Acetone 20% (formic acid)	Bochi et al. (2015)
Blackberry	Pomace	6.81-12.61 mg Cya/g dry extract	-	Methanol 80 % (0,05 acetic acid)	Cetojevic-Simin et al. (2017)
Raspberry	Pomace	2.32-4.28 mg Cya/g dry extract	70.0 g sample	Methanol 80 % (0,05 acetic acid)	Cetojevic-Simin et al. (2015)
Pomegranate	Peel	0.55 mg Cya/g extract	5,0 g sample	Acetone	Abid et al. (2017)
Blueberry	Pomace	13.4 mg Cya/g extract	-	Methanol	Correia et al. (2017)

- Cya, cyanidin equivalent.

3. Application of fruit by-products in meat products

Quality loss for non-microbiological causes in meat products was always one of the biggest concerns in the preservation of sensory properties of the meat product, because lipid and protein compounds of meat products are easily susceptible to oxidative damage, which may affect color, taste, texture and nutritional value of meat products (Silva et al., 2018). Oxidative reactions bring technological problems during processing and storage, and digestive problems (Hyun-Wook et al., 2018; Pomélie et al., 2018).

Sodium chloride, commonly known as salt, is a product widely used in the meat industry to ensure conservation of the meat matrix and in the extraction of proteins to obtain high quality products. However, at the same time, it brings technological benefits, accelerates oxidative reactions in the meat matrix, since it is a pro-oxidant (Jiang et al. 2019). The use of antioxidant compounds from fruit processing by-products are an alternative to decrease or eliminate the use of synthetic antioxidants used in the meat industry, in order to obtain healthier reformulated meat products with oxidative stability for much longer (Cunha et al., 2018).

Meat products are a source of nutrients, with water activity between 0.83-0.98, making microbial growth propitious. Meat industry uses additives such as sodium chloride, nitrites, phosphates, parabens, among others, however, not all antimicrobial compounds used in it are safe, and may have allergen and carcinogenic potential (Maher et al., 2018; Simpson, & Sofos, 2009). The use of antimicrobial compounds from fruit processing by-products are an alternative to decrease or eliminate the use of antimicrobials used in the meat industry that are allergen and carcinogenic potential (Andrés et al., 2017).

The addition of dietary fibers from fruit processing by-products to reformulate meat products can provide new functional properties, such, reduced weight loss and shrinkage by cooking, influences on viscosity and texture, and acts as fat substitute (Guedes-Oliveira et al.,

2016). Moreover, studies suggest that its consumption is beneficial to health, reducing the risk of digestive and cardiovascular diseases (Crowe et al., 2012; Resende, Franca, & Oliveira, 2019).

In addition, fruit processing by-products have other functional additives that interest the meat industry, such as dyes, acting as a color reinforcer, and that, if properly explored, can help imitating curing color in reformulated meat products (Savadkoohi, Hoogenkamp, Shamsi, & Farahnaky, 2014). Table 5 shows the effects of adding fruit processing by-products in the preparation of meat products

4. Technological challenges in the application of fruit by-products in meat products

The use of fruit processing by-products to reformulate meat products is one of the alternatives to improve agroindustry sustainability and overcome technological challenges in obtaining *Clean Label* foods (Kowalska et al., 2017). However, there are factors that must be considered, such as the application of heat treatment and acidification. The application of heat during the cooking of some meat products with addition of fruit by-products might decrease the antioxidant capacity and cause unwanted changes in color, because there are a few anthocyanins dyes that are sensitive to heat (Hernández-Herrero, & Frutos, 2014). In addition, the pH of the meat matrix will directly affect the color shade of these dyes, and color changes might occur during storage time (Arancibia-Avila et al., 2012).

The addition of fruit by-products in reformulated meat products may imply in the addition of allergen food proteins from fruit by-products (peel, seed, fruit remains), and that may be responsive for anaphylactic reactions, which can lead to disorders that mainly affect the skin and digestive tract. Several cases of people with allergic reactions by ingesting some fruit by-products have been reported in recent years (Barre, Simplicien, Benoist, Rougé, 2018; Boyano-Martínez, Pedrosa, Belver, Quirce, & García-Ara, 2013).

Table 5. Fruit by-products added in several meat products and the effects achieved

Meat product	By-product	Effect	Source
Beef meatballs	Pomegranate peel	Color, antioxidant activity, antimicrobial activity	Morsy et al. (2018)
	Pomegranate peel	Antioxidant activity, prolongs shelf life	Turgut, Işıkçı, & Soyer (2017)
Goat meatballs	Pomelo peel	Antioxidant activity, prolongs shelf life	Nishad et al. (2018)
Chorizo	Grape seed	Antioxidant activity	Lorenzo et al. (2013)
Beef burger	Grape marc	Antioxidant activity	García-Lomillo et al. (2017)
	Apple peel	Antioxidant activity	Sabally et al. (2016)
	Pineapple pomace	Cooking characteristics, texture profile	Selani et al. (2016)
Chicken burger	Cashew pomace	Cooking characteristics, fat substitute	Guedes-Oliveira et al. (2016)
	Plum peel	Color, antioxidant activity, texture profile	Basanta et al. (2018)
Lamb burger	Tomato pomace	Color, antioxidant activity, texture profile, antimicrobial activity	Andrés at al. (2017)
Pork burger	Passion fruit albedo	Fiber content, texture profile, cooking characteristics, antioxidant activity	López-Vargas et al. (2014)
	Pitaya peel	Antioxidant activity, texture profile	Cunha et al. (2018)
	Guarana seed	Color, antioxidant activity	Pateiro et al. (2018)
Bologna sausage	Jabuticaba peel	Color, antioxidant activity, antimicrobial activity	Almeida et al. (2015)
Salami	Grape seed	Antioxidant activity	Aquilani et al. (2018)
Frankfurt sausage	Tomato pomace	Color, dietary fiber content, texture profile	Savadkoohi et al. (2014)
Restructured fish meat	White grape marc	Antioxidant activity, dietary fiber content, extends shelf life	Sánchez-Alonso et al. (2008)

Another point to evaluate is the risk of ingestion of pesticide residues used during the combat of agricultural pests, concentrated largely in the peel of fruit. Studies suggest that the indirect ingestion of pesticide residues increases the incidence in the development of cancer and degenerative neurological diseases (Han et al., 2011; Sabarwal, Kumar, & Singh, 2018).

Another challenge is that phenolic compounds can cause a bitter taste and sensation of astringency in the reformulated meat product. Therefore, the disproportional addition of fruit byproducts can modify the characteristic color, flavor, aroma and texture of the meat products (Dinnela, Recchia, Tourila, & Monteleone, 2011). Moreover, studies indicate that there is interaction between phenolic compounds and other food, which may increase the droplet size in the emulsion system (Jakobek, 2015).

However, in order to benefit best from the junction of bioactive and functional properties of fruit processing by-products with the development of new reformulated meat products, it is still necessary to research how functional and bioactive properties of fruit by-products relate better with each meat product. The several formulations, conditions and preparation processes, as well as storage conditions, will provide different effects in the fruit by-product.

5. Alternatives to optimize the application of fruit by-products in meat products.

For a better conservation of bioactive compounds in meat products with addition of fruit by-products, they should not be submitted to high temperature treatments for long periods. However, the lower the cooking temperature, the better will be preserved the dyes of fruit by-products (Arancibia-Avila et al., 2012).

The heat treatment can decrease the quantity of allergenic proteins, as Sanchiz et al. (2018) suggest. The authors subjected cashew and pistachio seeds to heat treatment (130°C for 30 minutes at 2.56 atm). However, in this study, the authors reported that not all allergenic proteins

were eliminated. Thus, it is still necessary to research new ways to eliminate allergenic proteins that some fruit by-products have, but, for now, a correct information of ingredients in the reformulated meat products with addition of fruit by-products can prevent any kind of disorder against the consumer's health.

The perception of bitter taste and the sensation of astringency of fruit processing by-products is due to the fact that fruit by-products are rich in phenolic compounds, and the interaction of phenolic compounds (PC) with saliva proteins (SP) forms a complex where the protein is captured and precipitated, providing the sensation of astringency and bitterness in the mouth. Several studies have reported that the change in conformation of the complex PC-SP-PC blocks the perception of astringency and bitterness in the mouth (Soares, Mateus, Freitas, 2012).

The use of some ingredients and food additives, which is common in the food industry, such as powdered milk, sucrose, sucralose, pectin and carboxymethylcellulose, have the potential to restructure the PC-SP-PC complex, blocking the sensation of astringency in the mouth (Ares, Barreiro, Deliza, & Gámbaro, 2009). Meat product reformulations with addition of fruit by-products, if the technological challenge is the astringent and bitter taste due to the presence of phenolic compounds, may consider the use of the described additives to increase the acceptability of the meat product with addition of fruit by-products.

6. Conclusion

The use of fruit processing by-products is of great importance due to the high content of bioactive compounds of interest for food industry to contribute with economic and environmental sustainability, and with the increase of food security in the world. The consumer's demand for food with natural ingredients, but safe and stable for consumption means that the use of food processing by-products in the reformulation of meat products is an alternative to supply the needs of the fruit

processing and meat industry. However, challenges such as the stability of bioactive compounds in the meat matrix during processing and storage of the meat product, the change in sensory characteristics when adding fruit by-products with phenolic compounds, which can change the color, aroma, texture and flavor characteristic of the traditional meat product, the dissemination of allergenic proteins or pesticide residues from fruit by-products, among other challenges, must be overcome to unify both raw materials. Therefore, there is still a need for more researches to find possible solutions to overcome these challenges. However, obtaining meat products with addition of fruit by-products has a promising future.

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CHAPTER 2

Effect of the application of raspberry (*Rubus idaeus*) and blackberry (*Rubus brasiliensis*) pulp processing by-products on color, texture profile, antioxidant activity, and cooking characteristics in chicken burger

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Effect of the application of raspberry (*Rubus idaeus*) and blackberry (*Rubus brasiliensis*) pulp processing by-products on color, texture profile, antioxidant activity, and cooking characteristics in chicken burger

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ABSTRACT

Although fruit by-products present high technological potential, in general, they are not valued by the food industry. In this context, the objective of this work was to characterize by-products of pulp processing of raspberry (RB) and blackberry (BB), evaluating the effect of their application on physicohemical, cooking characteristics, antioxidant capacity, and texture profile in chicken burger formulations. Treatments: two controls, CF1 (with addition of sodium ascorbate) and CF2 (without addition of antioxidant), and F1 and F2, with addition of 2% RB and 2% BB, respectively. On average, the addition of RB and BB to chicken burger increased the total lipids and total fiber contents by 25.95% and 1.1%, respectively; changed color of burgers and increased the total phenolic content and activity antioxidant by 99.5% and 28.3%, respectively. Besides, decreased cooked loss by 6.98%, diameter shrinkage by 14.19%, and decreased the hardness of the samples by 5.09%.

Keywords: By-products, bioactive compounds, phenolic compounds, natural antioxidant, dietary fiber, chicken burger.

1. Introduction

Berries are small fruits, widely cultivated worldwide, with high content of bioactive compounds, such as phenolic, ascorbic acid and others (Souza, Pereira, Silva, Lima, Pio & Queiroz,

2014). Blueberry, blackberry, raspberry and strawberry are in this group. Studies suggest that the consumption of berries as part of the human diet can bring important health benefits, since they have antioxidant, anti-inflammatory, anticancer, antihypertensive and cardioprotective properties (Bowen-Forbes, Zhang & Nair, 2010; Liobikas, Skemiene, Trumbeckaite & Borutaite, 2016).

However, due to the fact berries are highly perishable, they are usually processed as pulp or juice, producing significant volumes of fruit by-products, such as peels, seeds, stalks and small amounts of other parts of the fruit. These by-products account for about 20% to 30% of the weight of the fruit, and their use is challenging, since they are unsuitable for animal feed purposes because of low protein content and low pH (Salaheen, Peng & Biswas 2015). Nevertheless, berries by-products are rich in bioactive compounds, which are found in greater proportion in peels and seeds, preserving the same health beneficial properties as the fruits themselves. Moreover, they are source of natural dyes, dietary fibers, compounds with antioxidant and antimicrobial activity, among other compounds of interest to the food industry (Biswas, Kumar, Bhosle, Sahoo & Chatli, 2011; Cetojevic-Simin et al., 2015, Salaheen, Nguyen, Hewes & Biswas, 2014).

Chicken meat is one of the most widely accepted type of meat in the world, and it is characterized by being leaner and has a higher quantity of phospholipids compared to other types of meat, which in turn, with presence of unsaturated fatty acids (Wilson, Pearson, & Shorland, 1976). One way of consuming chicken meat is as burger, which is one of the most consumed meat products worldwide (Feiner, 2006).

Nonetheless, phospholipids present in chicken meat, processing conditions as grinding and addition of salt, freezing rate, storage time and conditions are factors that accelerate oxidation reactions during cooking, developing rancid flavors and aromas (Gheisari, & Motamedi, 2010; Soyer, Özalp, Dalmış, & Bilgin, 2010). Using synthetic antioxidants as butylhydroxyanisole (BHA), butylhydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) is an alternative to delay

or inhibit oxidative reactions, but their use in food may be related to toxicological effects (Sarafian, Kouyoumjian, Tashkin, & Roth, 2002).

The application of berries pulp processing by-products to reformulate chicken burgers is an alternative that can supply the need for making the most of it, adding value to the by-products from fruit pulp industry, and obtaining more stable chicken burgers throughout storage time (Gouw, Jung & Zhao, 2017). The objective of this work was to characterize of by-products of pulp processing of raspberry (*Rubus idaeus*) and blackberry (*Rubus brasiliensis*), evaluating the effect of their application on physicohemical and functional properties of chicken burger.

2. Material and methods

2.1 Obtaining and packaging raw materials

By-products of pulp processing of raspberry (*Rubus idaeus*) and blackberry (*Rubus brasiliensis*), consisting of peels and seeds, were obtained from Sítio do Bello, located in Paraibuna, São Paulo (S 23º 23', W 45º 39'), in November 2016. Soon after the by-products were obtained, they were packed in plastic bags protected from light, frozen and then transported to the Laboratory of Fruit, Vegetables and Confectionery Products of the State University of Campinas, where remained frozen at -18 ºC until drying. The by-products were dried by convection in an air circulation oven (Marconi MA035, Brazil) at 40 ºC for 48 hours, and ground in a Grindomix GM200 Knife Mill (Restch, Germany) until obtainment of ≤0.21 mm particles (65 mesh). Then, by-products were stored at -18°C in vacuum-packed polyethylene bags until the chicken burgers were made.

Refrigerated chicken meat, particularly chicken breast fillet, was obtained in a commercial establishment in Campinas-SP. The meat was transported in plastic bags to the Laboratory of Meat and Meat Products of the State University of Campinas, and maintained under refrigeration. Meat pH was measured to verify the good condition of the raw material; then it was

manually chopped and ground using a 5-mm grinding disc meat chopper (CAF model 22STB, Brazil), and maintained at -2 ºC until the next day, when the chicken burgers were made.

2.2 Treatments

After preliminary tests to determine the percentage of addition of raspberry and blackberry by-products in chicken burger, four formulations were prepared, namely: control formulation with addition of sodium ascorbate (CF1); control formulation without addition of antioxidant (CF2); formulation with addition of 2% raspberry by-products (F1), and formulation with addition of 2 % blackberry by-products (F2), as described in Table 1.

Table 1. Formulation of chicken burger treatments in percentages

Ingredients	CF1	CF2	F1	F2
Chicken breast	73	73	73	73
Canola oil	5	5	5	5
Water	18.15	18.2	16.2	16.2
Raspberry by-products	0	0	2	0
Blackberry by-products	0	0	0	2
Sodium chloride	2	2	2	2
Sodium ascorbate	0.05	0	0	0
Maltodextrin	1.8	1.8	1.8	1.8
Total	100	100	100	100

2.3 Chicken burger processing

The chicken burgers were elaborated in the pilot plant of the Laboratory of Meat and Meat Products of the School of Food Engineering, State University of Campinas, in refrigerated environment at temperature lower or equal to 10 ºC. The preparation was performed in 3-kg batches in homogenizer (Filizola - Sire, Brazil). The process was performed in triplicate.

For CF1 and CF2, ground chicken meat, sodium chloride and water were mixed for 60s; then maltodextrin was added, and the mixture was homogenized for more 60s. Canola oil was added and mixed for additional 20s. In the case of CF1, sodium ascorbate was added after maltodextrin and before canola oil, and the mixture was homogenized for more 20s. For F1 and F2, raspberry by-products (RB) and blackberry by-products (BB) powder were respectively added in the first stage of preparation, along with ground chicken meat, sodium chloride and water.

Formulations were stored between 0 °C and 1 °C for about one hour, and then, chicken burgers of 100 ± 5 g and 100 mm in diameter were made using a burger maker (Müller, Brazil). The chicken burgers were put in low density polyethylene bags, proper for meat products, and frozen at -18°C for about 8 hours to take shape. Then, CF1, CF2, F1 and F2 were stored at -18°C in vacuum transparent polyethylene bags for up to 60 days for performance of lifespan studies at 0, 15, 30, 45 and 60 days of storage.

Raw chicken burgers (0, 15, 30, 45 and 60 days) stored at -18°C were cooked in electric plate (Sire, Brazil) at 175 °C for 7 minutes, until reaching 75°C internal temperature. After cooking, the samples were cooled to 25 °C for analysis.

2.4 Analytical determinations to characterize fruit by-product

2.4.1 Centesimal composition

Nitrogen content (method 920.152), ashes (method 940.26), total and insoluble fibers (method 958.29), and moisture content (method 920.151) were determined according to AOAC (2006). Protein content was estimated by multiplying the amount of Nitrogen by 6.25. Lipid content was determined by the method of Bligh & Dyer (1959), and carbohydrate content was calculated by difference. Determinations were performed in triplicate.

2.4.2 Physicohemical characteristics

The potential of hydrogen (pH) was determined by obtaining the filtrate of fruit by-product powder with deionized water (10:90 g/mL), and pH value was read using a calibrated pH meter (Digimed, DM-20, USA). To determine the titratable acidity, a basic solution of 0.1M NaOH was used; after alteration of color, NaOH consumption was measured and acid value calculated as a percentage of citric acid. The determination of water activity (Aw) was done by direct reading on AquaLab® 4TEV (Decagon, USA), which uses dew point and capacitance to measure this activity. Analyzes were performed in triplicate.

2.4.3 Objective Color

Objective color characteristics were determined by Colorquest II spectrophotometer (Hunter Associates Laboratory, USA) at six different points of the petri dish with RB and BB powder to obtain the values of L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness). Hue angle (h^o) and Chroma (C^*) parameters were calculated by employing the means of L^*, a^* and b^* values according to the methodology followed by Gouw et al. (2017). Parameters were calculated with Equations 1 and 2.

$$h^o = tan^{-1}(b^*/a^*) \quad (1)$$

$$C^* = (a^{*2} + b^{*2})^{0.5} \quad (2)$$

2.4.4 Water holding capacity

Following the methodology of Chen, Rubenthaler, Leung & Baranowski (1988), with some modifications, 1g of powder of fruit by-products on a dry basis was weighted and homogenized in a Nalgene tube with 20 ml of 3.5% saline solution to simulate the meat matrix. Then, the pH was adjusted to 6.2 with 0.1M NaOH solution or 0.1M HCl solution, and homogenized for 1 min on a vortex mixer. It was then left in shaking water bath at 85°C x 100 rpm for 15 min, and then centrifuged at 1735 g for 10 min. After water excess elimination, the wet fiber

weight was found, and the value of Water Holding Capacity (WHC) was obtained following Equation 3. Analysis was performed in triplicate, and the result was expressed in g of water/g of by-product on a dry basis.

$$WHC = \left(\frac{\textit{Wet fiber weight - sample weight on a dry basis}}{\textit{sample weight on a dry basis}}\right) \qquad (3)$$

2.4.5 Extraction of phenolic compounds

Extraction of phenolic compounds from the fruit by-products was performed in triplicate and conducted in two stages, as follows: first extraction with 40 mL of 50% methanol solution, and second extraction with 40 mL of 70% acetone solution. Both extractions were performed at room temperature during 60 min protected from light. The extracts were centrifuged at 1750 g for 15 min, and both supernatants were collected, mixed, supplemented with deionized water to 100 mL and stored in a glass vial protected from light at -18°C (Larrauri, Rupérez & Saura-Calixto, 1997). The concentrations of the extracts depended on obtaining acceptable values for absorbance readings.

2.4.6 Total monomeric anthocyanin pigment content

Total monomeric anthocyanin pigment content was calculated by using the differential pH method (Wrolstad & Giusti, 2001), with potassium chloride buffer solution (0.025 M, pH 1.0), and sodium acetate buffer solution (0.4M, pH 4.5). The concentrations of RB and BB extracts were 5% and 1%, respectively. Two aliquots of each extract were used and mixed with each buffer solution in a ratio of 1:4 (extract:buffer solution), allowed resting for 15 min, and then absorbance readings were performed at 510 nm and 700 nm on the Orion AquaMate 8000 UV-Vis Spectrophotometer (Thermo Scientific, USA). The diluted sample absorbance was calculated with Equation 4, and total monomeric anthocyanin concentration was calculated by the Equation 5.

$$A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5} \qquad (4)$$

$$TMA\ (mg/L) = (A \text{ x } MW \text{ x } DF \text{ x } 1000) / (\varepsilon \text{ x } 1) \qquad (5)$$

Where *TMA* is the total monomeric anthocyanin concentration, *A* is the diluted sample absorbance, *MW* is the molecular weight of cyanidin-3-glucoside (449.2 $g.mol^{-1}$), *DF* is the sample dilution factor, and ε is the molar absorptivity of cyanidin-3-glucoside (26 900 $L.cm^{-1}.mol^{-1}$).

The results were expressed in mg of cyanidin-3-glucoside equivalents/100 g of fruit by-products on a dry basis. Determination was performed in triplicate.

2.4.7 Total phenolic content

For the determination of total phenolic content, Folin-Ciocalteu colorimetric method (Singleton, Orthofer & Lamuela-Raventós, 1999) was applied. The concentration of the extract of fruit by-products was 0.1%. An aliquot of each extract was mixed with 10% solution of Folin-Ciocalteu Phenol Reagent, with maintenance of 1:5 (extract:solution) ratio. Then the 7.5% Sodium Carbonate solution was added to the mixture, with maintenance of 1:8 (extract:solution) ratio. After 120 min resting protected from light and at room temperature, absorbance readings were made at 760 nm on the Orion AquaMate 8000 UV-Vis Spectrophotometer (Thermo Scientific, USA). For the blank measurement, extractive solution (40:40:20, 50% methanol: 70% acetone: deionized water) was used. The absorbance readings of gallic acid solutions at different concentrations (0, 8, 16, 32, 48, 64 and 72 μg/mL) established the standard curve points to determine gallic acid curve equation. The results were expressed in mg of gallic acid equivalents/g of fruit by-products on a dry basis. Analysis was performed in triplicate.

2.4.8 Antioxidant activity

The antioxidant activity was determined by the $ABTS^{\bullet +}$ discoloration assay (Re, Pellegrini, Proteggente, Pannala, Yang & Rice-Evans, 1999). There was preparation of 2.45 mM

potassium persulfate solution; 7 mM ABTS (2,29-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) diluted solution, and 2 mM Trolox (6-hydroxy-2,5,7,8-tetramethrychroman-2-carboxylic acid) standard solution in different concentrations (20, 100, 500, 1000, 1500 and 2000 μM) to establish standard curve absorbance values. The standard curve reaction occurred by homogenizing 30 μL of Trolox standard solution in different concentrations with 3 mL of ABTS solution, incubated in water bath at 30°C for 25 min. Then, absorbance readings were made at 734 nm on the Orion AquaMate 8000 UV-Vis Spectrophotometer (Thermo Scientific, USA). For RB and BB extract reaction, the procedure was the same as for Trolox standard solution reaction, however with aliquots of 30 μL of 0.1% extracts. The results were expressed in μmol of Trolox equivalents/g of fruit by-products on a dry basis. Analysis was performed in triplicate.

2.5 Characterization of chicken burger

2.5.1 Centesimal composition

Total nitrogen determination analyzes (method 920.152) using factor 6.25, ashes (method 940.26) and moisture content (method 920.151) were performed following AOAC (2006) methods. Lipid content was determined by the method followed by Bligh & Dyer (1959). Cooked chicken burger analyzes were performed in triplicate.

2.5.2 Physicohemical characteristics

There was homogenization of 10 g of cooked chicken burger sample in deionized water (1:10 sample/water), and the potential of hydrogen (pH) was measured by using a previously calibrated pH meter (Digimed, DM-20, USA). Water activity (Aw) was determined by direct reading on AquaLab® 4TEV (Decagon, USA), which uses dew point and capacitance to measure this activity. Determinations were performed in triplicate.

2.5.3 Objective Color

The color was determined by the MiniScanTM XE (Hunter Associates Laboratory, USA) colorimeter at three different points of raw and cooked chicken burgers, and L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) were found. Readings were done in triplicate with three repetitions each. With the means of L^*, a^* and b^* values, Hue angle (h^o) and Chroma (C^*) parameters were calculated (Almeida et al., 2015) with Equations 1 and 2.

2.5.4 Extraction of phenolic compounds

The cooked chicken burger samples of 60-day storage were lyophilized at -45°C and 0.012 mbar on the Freeze Dryer Alpha 2-4 LD plus (Christ, Germany) for approximately 36 hours, then crushed in a mortar. Following the same methodology described in item 2.4.5, phenolic compounds were extracted with 5% chicken burger extract concentration. Extracts were stored in a glass vial protected from light at -18 °C (Larrauri et al., 1997). Extractions were done in triplicate.

2.5.5 Total phenolic content and antioxidant activity

To determine the total phenolic content and antioxidant activity of the cooked chicken burger samples, Folin-Ciocalteu colorimetric method (Singleton et al., 1999) and ABTS discoloration assay (Re et al., 1999) were applied, respectively, as described in items 2.4.7 and 2.4.8, although using the extracts obtained from the chicken burger samples. The results for determination of total phenolic content were expressed as mg of gallic acid equivalents/g of chicken burger on a dry basis, and the antioxidant activity was expressed in μmol of Trolox equivalents/g of chicken burger on a dry basis. Analyzes were performed in triplicate.

2.5.6 Cooking characteristics

Percentage of cooking loss (% CL) and percentage of diameter shrinkage (% DS) were determined by Equations 6 and 7. Analyzes were performed in triplicate.

$$\% CL = \frac{(uncooked\ burger\ weight - cooked\ burger\ weight)}{uncooked\ burger\ weight} \times 100 \quad (6)$$

$$\% DS = \frac{(uncooked\ burger\ diameter - cooked\ burger\ diameter)}{uncooked\ burger\ diameter} \times 100 \quad (7)$$

2.5.7 Texture profile

The TA-XT 2i texture analyzer (Texture Technologies Corp./Stable Micro Systems, UK) was used to measure hardness, elasticity, cohesiveness and gumminess of cooked chicken burger samples with 2.5 cm diameter and 2 cm thickness, according to Bourne (1978), quoted by Selani et al. (2016). The measurements were performed at room temperature, according to the procedures of the American Meat Science Association (AMSA), described by Claus (1995). The compression cycle was done in two consecutive times with a 5-second interval, with compression of 75% original thickness, and 6.6 mm/s pre-test speed, 3.3 mm/s test speed, and 6.6 mm/s post-test speed. A P-35 Probe (35 mm diameter) was used.

2.5.8 Statistical analysis

Data referring to the analysis of fruit by-products were evaluated by Student's t-test. Data on chicken burger treatments such as total anthocyanin pigment content, total phenolic content, antioxidant activity, objective color and texture profile were analyzed by using a General Linear Model (GLM) variance test, and other data were evaluated by analysis of variance by One-way (ANOVA). In both cases (GLM and One-way), the means were compared by Tukey test, considering level of 5% significance. Statistical analyzes were performed using Statistica software package (v7.0, StatSoft Inc., USA, 2004).

3. Results and discussion

3.1 Fruit by-products physicohemical characterization

Table 2 shows the centesimal composition of raspberry (RB) and blackberry (BB) by-products on a dry basis.

Table 2. Centesimal composition of raspberry (RB) and blackberry (BB) by-products

Content (g/100 g bdb)	By-products	
	RB	BB
Moisture	3.76±0.17[b]	5.39±0.17[a]
Lipids	20.61±0.59[a]	14.67±0.64[b]
Proteins[1]	9.55±0.10[b]	12.26±0.23[a]
Ashes	2.54±0.02[a]	2.41±0.01[b]
Carbohydrates[2]	63.54 ±0.68[a]	65.27 ±0.78[a]
Total fibers	54.41±0.03[a]	55.85±0.01[a]
Insoluble fibers	36.42±0.02[a]	24.95±0.03[b]

(1) Protein content calculated as nitrogen content x 6.25.
(2) Value estimated by difference.
bdb, by-products on a dry bases.
Different letters on the same line indicate difference between samples.
The table shows the values of means ± standard deviation.

RB and BB protein content was 9.55 and 12.26 g/100 g bdb, respectively, and RB and BB lipid content was 20.61 and 14.67 g/100 g bdb, respectively. In turn, protein content of grape wine making by-product was 10.25 g, and the lipid content was 15.28 g, both in 100 g bdb (Nayak, Bhushan, Rosales, Rodríguez-Turienzo & Cortina, 2018). On the other hand, the protein content of apple juice processing by-product was 7.25 g, and the lipid content was 2.45 g, both in 100 g bdb (Chen et al., 1988). RB lipid content was higher than of BB, and this difference is because RB is mainly composed of seeds (Gouw et al., 2017), increasing lipid content significantly (Radocaj, Vujasinovic, Dimic & Basic, 2014).

RB total fiber and insoluble fibers contents were 54.41 g and 36.42 g, respectively, in 100 g bdb. Other studies reported different values regarding content of total fibers and insoluble fibers in raspberry processing by-product. Gouw et al. (2017) reported values of 38.47 g and 38.13 g of total fibers and insoluble fibers, respectively, both in 100 g bdb, and Górecka, Pachołek,

Dziedzic & Górecka (2010) reported values higher than those found in this study, that is, 77.5 g and 75.0 g of total fibers and insoluble fibers, respectively, both in 100 g bdb. Differences in fiber content in the different studies may be related to fruit variety, cultivation site and environmental growth conditions (Pantelidis, Vasilakakis, Manganaris & Diamantidis, 2007).

BB total fiber and insoluble fiber contents were 55.85g and 24.95g, respectively, both in 100g bdb. When compared to other fruit processing by-products, the total fiber content reported by Chen et al. (1988) in apple juice by-product was 61.90 g/100 g bdb, and fiber and insoluble fiber contents reported by Peixoto et al. (2018) in wine making by-product were 60.5 g and 49.3 g, respectively, both in 100 g bdb. Reported total fiber content values are similar, but there is difference in the insoluble fiber content; the possible inequality may be due to the difference in the proportion of peel and seed between the analyzed samples, which will have effect on the centesimal composition values (Peixoto et al., 2018).

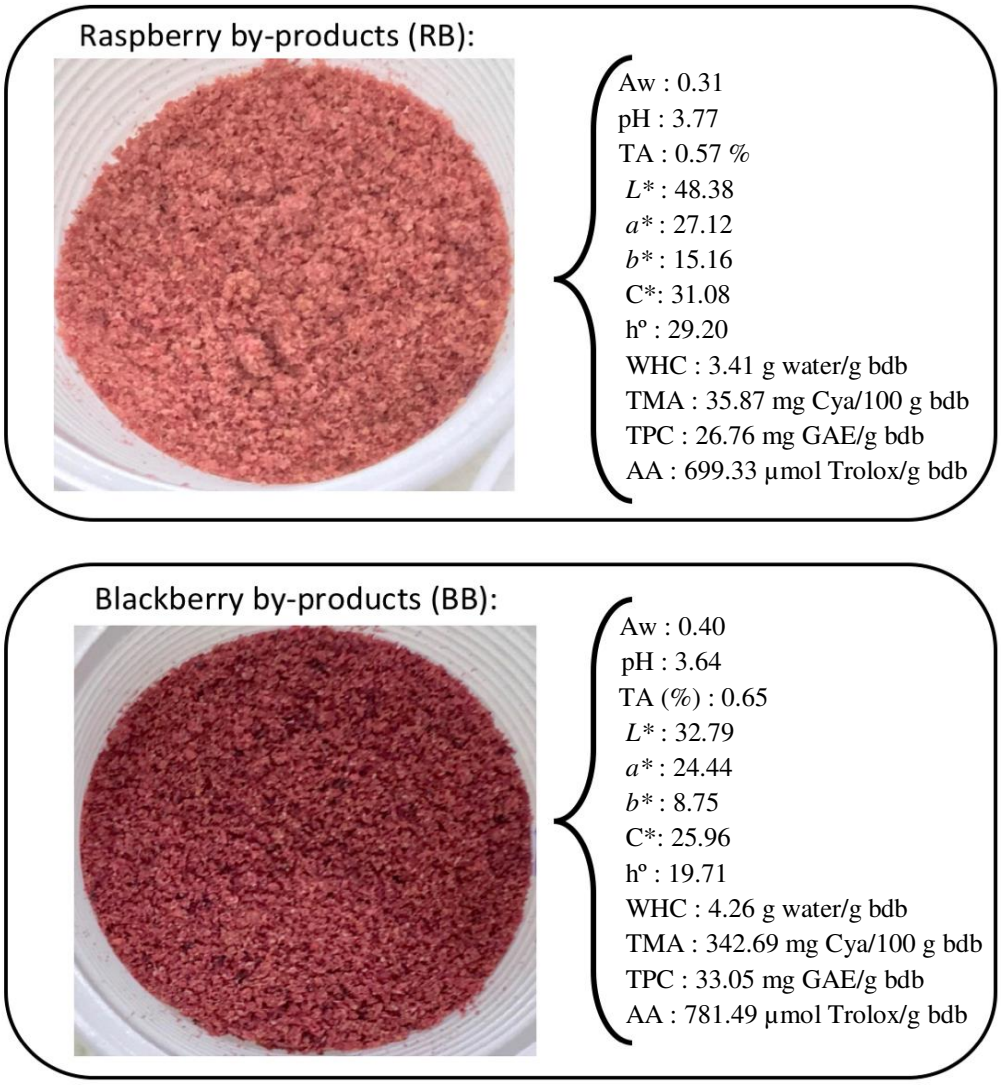
The results show raspberry and blackberry pulp processing by-products can be used as food ingredient to enrich both nutritional content and dietary fiber content beneficial to health as also mentioned by Biswas et al. (2011).

Figure 1 shows pH, Aw, objective color and water holding capacity values of raspberry and blackberry by-products.

There was no difference in pH values between RB and BB samples (3.77 vs. 3.64, respectively), however, there were differences in titratable acidity (0.57% vs. 0.65 %, respectively) and water activity (0.31 vs. 0.4, respectively) values.

Gurak, de Bona, Tessaro & Marczak (2014) reported pH value of jaboticaba pomace in powdered form of 3.74. On the other hand, Gouw et al. (2017), in turn, reported titratable acidity values of raspberry and blueberry juice processing by-products of 1.37% and 0.5%, respectively, both as a percentage of citric acid, and water activity values of RB and BB of 0.157 and 0.159,

respectively. Then, it is verified that the acidity percentage is closer to the acidity of blueberry by-product. In the study of Gouw et al. (2017), raspberry and blueberry were dried at 110°C for 3 hours and then ground to 0.5 mm particles. In this study, drying was performed at 40°C for 48 hours, and grinding was performed until ≤ 0.21mm particles.



Aw, water activity; TA, titratable acidity; *L**, lightness; *a**, redness/greenness; *b**, yellowness/blueness; C*, saturation or intensity; hº, hue angle; WHC, water holding capacity; TMA, total monomeric anthocyanin pigment content; TPC, total phenolic content; AA, antioxidant activity; bdb, by-product on a dry basis; Cya, cyanidin equivalents; GAE, gallic acid equivalents.

Figure 1. Physicochemical characteristics, objective color and water holding capacity of raspberry by-product (RB) and blackberry by-product (BB).

Pantelidis et al. (2007) reported that differences in physicochemical characteristics in the same fruit may be due to variety differences and fruit storage conditions.

*L**, *a** and *b** values were statistically different between the samples. RB color was brighter and directed to redness and yellowness, and BB color was oriented to the opposite direction. Regarding color saturation, RB had a more saturated color than BB, and in relation to the hue angle, RB got closer to bluish-red, and BB was orientated to yellow (Wrolstad, Durst & Lee, 2005).

The values for hue angle and saturation of RB were 29.20° and 31.08, respectively. Regarding raspberry juice processing by-product, Gouw et al. (2017) reported hue angle and saturation values of 60.17° and 31.44, respectively, and the difference in color values may be attributed to differences in sample drying conditions. According to Fischer, Carle & Kammerer (2013), application of high temperatures during drying of fruit by-products may degrade color compounds.

BB water holding capacity was higher than that of RB (4.26 g vs. 3.41 g w/g of bdb, respectively). Gouw et al. (2017), reported water holding capacity (WHC) values of raspberry and blueberry by-products of 7.71 g and 8.29 g w/g of bdb, respectively. The difference in WHC values may be attributed to differences in sample grinding conditions, and according to Biswas et al. (2011), water holding capacity is one of the properties of fibers, however, depends on the types of fibers, its concentration, temperature, pH and presence of ions.

Total anthocyanin and total phenolic contents in RB was 35.87 mg Cya/100 g bdb, and 26.76 mg GAE/g bdb. On the other hand, Pantelidis et al. (2007) reported values of 35.1-49.1 mg Cya/100 g of raspberry fresh weight for total anthocyanin content, and values between 10.52-24.94 mg GAE/g of raspberry on a dry basis. Cetojevic-Simin et al. (2015) determined total anthocyanin

of 2.32-4.28 mg Cya/g RB dry extract, and total phenolic content values of 26.3-43.7 mg GAE/g RB dry extract.

Total anthocyanin and total phenolic contents found in BB were 342.69 mg Cya/100 g bdb, and 33.05 mg GAE/g bdb. Pantelidis et al. (2007) reported total anthocyanin content values between 125.6-152.2 mg Cya/100 g of blackberry fresh weight, and 17.03-23.49 mg GAE/g of blackberry on a dry basis. Cetojevic-Simin et al. (2017) reported total anthocyanin content values between 5.90-11.49 mg Cya/g BB dry extract, and total phenolic content values between 75.50-88.28 mg GAE/g BB dry extract.

Gurak et al. (2014) reported total anthocyanin and total phenolic content values of jaboticaba by-products (3.92 mg Cya/g bdb and 43.39 mg GAE/g bdb, respectively). On the other hand, Babbar, Oberoi, Uppal & Patil (2011) reported total phenolic content values of grape seed of 37.4 mg GAE/g bdb. In contrast, Nayak et al. (2018) reported total phenolic content of grape pomace between 4.28-8.02 mg GAE/g bdb. Pantelidis et al. (2007) concluded that harvest period, varieties, solvents and extraction conditions, particle size at the time of extraction, and sample drying conditions directly interfere in phenolic compound content.

The antioxidant capacity applied by the ABTS discoloration assay of RB and BB were 699.33 μmol and 781.49 μmol of Trolox equivalents/g bdb, respectively. Babbar et al. (2011) reported values of 42.23 meq of Trolox/g of grape by-product (seed) on a dry basis. On the other hand, Souza et al. (2014) reported antioxidant capacity-ABTS in raspberry and blackberry fruits of 6.27 and 13.23 μmol Trolox equivalents/g fresh weight, respectively.

The determination of phenolic content and antioxidant activity in raspberry and blackberry pulp processing by-products is extremely important for estimation of the antioxidant potential and their possible applications in the reformulation of foods to retard or inhibit oxidative reactions (Viljanen, Kylli, Kivikari, & Heinonen, 2004).

3.2 Characterization of chicken burger

3.2.1 Centesimal composition

Table 3 shows the values of analysis of centesimal composition, pH and water activity of freshly processed samples.

Table 3. Centesimal composition, pH and water activity of control chicken burgers (CF1 and CF2), raspberry by-products (F1) and blackberry by-products (F2)

	Cooked chicken burger samples			
Content	CF1	CF2	F1	F2
Moisture*	74.6±0.13[a]	73.61±0.46[b]	71.52±0.14[c]	71.33±0.20[c]
Lipids*	4.90±0.05[c]	5.85±0.04[b]	6.90±0.11[a]	6.64±0.19[a]
Proteins*	14.5±0.91[a]	14.98±0.08[a]	14.90±0.45[a]	14.85±0.34[a]
Ashes*	2.71±0.01[b]	2.74±0.02[b]	2.71±0.02[b]	2.82±0.0[a]
pH	5.97±0.02[a]	6.07±0.08[a]	5.77±0.06[b]	5.75±0.06[b]
Water activity	0.98±0.01[a]	0.97±0.0[a]	0.97±0.0[a]	0.97±0.0[a]

* By g/100g. Equal letters on the same line indicate no difference between the samples, and different letters indicate there was difference. The table shows the values of means ± standard deviation.

Addition of RB and BB caused no difference in protein content in cooked chicken burger treatments; this may be because F1 and F2 treatments have lower humidity than CF1 e CF2 treatments. On the other hand, López-Vargas, Fernández-López, Pérez-Álvarez & Viuda-Martos (2014) reported that there was statistical difference in protein content in cooked pork burger added with by-product of yellow passion fruit albedo, where, to higher by-products addition, lower protein content.

Lipid content was higher in F1 and F2, differing significantly from CF1 and CF2. Addition of RB and BB increased lipid content due to the presence of oils from the seeds (Radocaj et al., 2014). However, they decreased the pH value of chicken burgers, and this may be because

grounding RB and BB releases fatty acids from the seeds, increasing chicken burger acidity (Radocaj et al., 2014).

CF1 treatment (with sodium ascorbate) showed a significant difference in relation to values of higher moisture mean – 74.6% – when compared to other treatments. This may be because the sodium ascorbate in contact with water dissociates, slightly increasing the acidity, decreasing pH value, and increasing water holding capacity (Feiner, 2006).

Alves, Marques, Carvalho, Pinheiro, Ramos & Corrêa (2017) added 1.5% powdered jabuticaba peel (particle size ≤0.5 mm) to the preparation of restructured hams, reporting that the this addition decreased total lipid content from 3.27% to 2.87%, and increased protein content from 11.47% to 12.12%. The pH value decreased significantly, from 6.03 to 5.67, and there was no difference in water activity between treatments.

3.2.2 Objective Color

The color of raw and cooked chicken burger was evaluated to determine whether there was difference during storage time. The results are shown in Table 4.

Raw CF2 treatment (control without addition of antioxidant) was the one that presented the highest increase in *b** and Chroma values throughout storage time, which indicates increased yellow intensity when compared with CF1 treatment (control with sodium ascorbate), as well as, increased saturation. This may mean that CF2 was more sensitive to lipid and protein oxidation during the 60-day storage, since sodium ascorbate has antioxidant effect (Feiner, 2006).

Raw F2 treatment (with addition of BB) was the one who had the highest increased of *a** throughout storage time when compared with F1 treatment (with addition of RB). Moreover, by relating this information to the results of total anthocyanin and phenolic content reported in this study, as well as the amount of polyunsaturated fatty acids in RB reported by Radocaj et al. (2014).

Table 4. Measurement of *L**, *a**, *b** objective color in chicken burger throughout storage time

Measurement	Storage time (days)	Raw chicken burger samples			
		CF1	CF2	F1	F2
*L**	0	75.05±2.28^{aA}	76.18±2.47^{aA}	67.04±2.5^{bA}	56.69±1.29^{cA}
	15	73.95±1.34^{aAB}	74.31±3.34^{aAB}	62.74±2.32^{bB}	56.44±0.49^{cA}
	30	72.80±2.16^{aB}	75.72±4.40^{aAB}	61.71±2.83^{bB}	51.05±1.78^{cC}
	45	73.71±0.81^{aAB}	72.28±3.27^{aB}	60.66±2.1^{bB}	54.39±2.0^{cB}
	60	60.46±1.78^{bC}	63.66±1.40^{aC}	55.02±1.76^{cB}	43.57±0.79^{dD}
*a**	0	6.10±0.62^{cA}	6.59±0.38^{bcA}	7.05±0.88^{bB}	8.67±0.81^{aC}
	15	5.68±0.15^{bA}	6.25±0.78^{bA}	9.12±0.52^{aA}	9.38±0.33^{aBC}
	30	5.77±0.21^{cA}	5.90±0.70^{cA}	8.59±0.35^{bA}	10.49±0.56^{aA}
	45	5.61±0.32^{bA}	6.36±0.66^{bA}	9.06±0.38^{aA}	9.58±0.84^{aABC}
	60	5.95±0.93^{cA}	6.17±0.41^{cA}	9.07±0.55^{bA}	9.90±0.68^{aAB}
*b**	0	13.64±1.58^{aC}	13.72±1.46^{aD}	9.78±2.20^{bC}	5.78±0.82^{cB}
	15	14.91±0.54^{abC}	16.14±0.42^{aC}	13.69±2.02^{bAB}	7.23±0.45^{cA}
	30	17.29±0.27^{aB}	16.27±1.44^{aBC}	12.63±1.72^{bB}	7.34±0.11^{cA}
	45	17.39±0.63^{aB}	17.53±1.35^{aB}	13.52±2.49^{bB}	6.77±0.52^{cA}
	60	19.12±1.85^{bA}	20.55±0.50^{aA}	15.86±0.93^{cA}	5.66±0.56^{dB}
Chroma	0	14.94±1.67^{aC}	15.22±1.43^{aD}	12.12±1.97^{bC}	10.42±1.13^{bC}
	15	15.95±0.54^{bC}	17.33±0.18^{aC}	16.50±1.56^{abAB}	11.84±0.54^{cAB}
	30	18.22±0.30^{aB}	17.34±1.08^{aC}	15.32±1.21^{bB}	12.81±0.46^{cA}
	45	18.27±0.69^{aB}	18.67±1.01^{aB}	16.34±2.03^{bB}	11.73±0.98^{cAB}
	60	20.03±2.00^{bA}	21.46±0.54^{aA}	18.27±1.06^{cA}	11.41±0.81^{dBC}
Hue angle (º)	0	65.87±1.05^{aD}	64.21±1.85^{aC}	53.49±6.86^{bB}	33.54±1.50^{cC}
	15	69.13±0.68^{aC}	68.83±2.89^{aB}	55.95±5.17^{bAB}	37.57±0.79^{cAB}
	30	71.56±0.52^{aB}	69.84±4.11^{aAB}	55.43±5.03^{bAB}	35.02±1.53^{cA}
	45	72.14±0.40^{aAB}	69.90±3.52^{aAB}	55.57±5.80^{bAB}	35.28±0.39^{cAB}
	60	72,78±1,40^{aA}	73.30±0.96^{aA}	60.24±0.71^{bA}	29.73±1.77^{cBC}

Measurement	Storage time (days)	Cooked chicken burger samples			
		CF1	CF2	F1	F2
*L**	0	75,65±4,08^{aA}	76.37±3.07^{aA}	50.96±7.35^{bA}	43.94±5.17^{cA}
	15	67.25±4.05^{aB}	63.27±5.57^{aB}	51.92±8.37^{bA}	48.03±5.09^{bA}
	30	63.13±6.02^{aB}	59.20±6.92^{aBC}	50.13±7.41^{bA}	47.13±3.35^{bA}
	45	69.12±4.91^{aAB}	65.66±6.0^{aB}	49.03±6.49^{bA}	46.72±5.28^{bA}
	60	53.04±6.85^{aC}	54.40±5.23^{aC}	52.17±7.54^{aA}	43.39±2.98^{bA}
*a**	0	4.98±2.36^{bC}	4.98±1.87^{bcC}	11.12±2.57^{aA}	12.81±0.82^{aA}
	15	7.88±1.56^{bBC}	11.77±3.07^{aB}	10.30±1.70^{abA}	11.90±2.19^{aA}
	30	9.45±2.50^{bB}	13.57±3.52^{aAB}	9.76±1.70^{bA}	11.96±1.57^{abA}
	45	7.25±1.73^{bBC}	10.74±3.21^{aB}	10.66±1.24^{aA}	12.16±1.92^{aA}
	60	16,26±3,84^{aA}	16.57±3.22^{aA}	10.74±1.61^{bA}	12.69±1.88^{bA}
*b**	0	21.63±5.08^{aC}	22.89±3.35^{aC}	20.12±6.32^{aA}	21.72±1.44^{aA}
	15	25.41±2.52^{bBC}	31.51±3.89^{aB}	15.62±4.48^{cA}	19.97±3.24^{cA}
	30	28.66±3.06^{bAB}	34.69±4.12^{aAB}	16.19±3.44^{cA}	19.64±2.80^{cA}
	45	24.36±2.79^{bC}	30.35±3.40^{aB}	16.87±5.15^{cA}	19.25±3.18^{cA}
	60	32.73±2.90^{bA}	37.02±2.92^{aA}	20.16±3.92^{cA}	21.81±3.46^{cA}
Chroma	0	22.22±5.49^{aC}	23.45±3.66^{aC}	23.12±6.32^{aA}	25.24±1.34^{aA}
	15	26.62±2.84^{bBC}	33.67±4.67^{aB}	18.88±3.96^{cA}	23.25±3.89^{bcA}
	30	30.21±3.60^{bB}	37.29±5.08^{aAB}	18.99±3.34^{cA}	23.00±3.18^{cA}
	45	25.43±3.13^{bC}	32.26±4.17^{aB}	20.10±4.65^{cA}	22.78±3.65^{bcA}
	60	36,65±3,87^{aA}	40.60±3.86^{aA}	22.91±3.81^{bA}	25.24±3.89^{bA}
Hue angle (º)	0	77,62±2,79^{aA}	78.05±2.70^{aA}	59.70±7.79^{bA}	59.43±2.22^{bA}
	15	72.91±1.88^{aB}	69.80±2.83^{aBC}	55.57±8.59^{bA}	59.28±0.96^{bA}
	30	71.99±3.01^{aB}	68.95±2.93^{aBC}	58.44±6.03^{bA}	58.62±1.20^{bA}
	45	73.62±2.30^{aB}	70.92±3.99^{aB}	56.30±7.89^{bA}	57.67±1.84^{bA}
	60	63.82±4.51^{abC}	66.08±2.83^{bC}	61.52±5.03^{bA}	59.77±1.18^{bA}

*L**, lightness; *a**, redness/greenness; *b**, yellowness/blueness; Chroma, saturation. Equal horizontal lower cases indicate no difference between the samples, and vertical capital letters indicate no difference regarding storage time. The table shows the values of means ± standard deviation.

This may have indications that raw F2 treatment was more sensitive to oxidative reactions throughout the storage time, both for lower content of antioxidant compound and for higher content polyunsaturated fatty acids (Soyer et al., 2010).

Cooked CF1 and CF2 treatments showed significant changes in *L**, *a**, *b**, Chroma and Hue angle values when compared with treatments with addition of RB and BB, which may mean lipid and protein oxidation reactions during storage time (Soyer et al., 2010). The color of F1 and F2 samples remained stable throughout storage time, possibly due to the presence of antioxidant compounds in RB and BB, which may have prevented significant changes in *L**, *a**, *b**, Chroma and hue angle values in chicken burgers as a consequence of lipid and protein oxidation (Radocaj et al., 2014).

Antioxidant compounds of RB and BB were more effective when cooking the frozen chicken burgers, due to chemical reactions happen in function of availability of free water to propagate oxidative reactions (Utrera, Morcuende & Estévez, 2014). Thus, the data presented for color confirm the effectiveness of the antioxidant compounds present in chicken burgers with addition of RB and BB.

Soyer et al. (2010) reported that freezing accelerates lipid and protein oxidation in chicken meat, triggering several reactions of polyunsaturated fatty acid decomposition, and reducing meat lightness. García-Segovia, Andrés-Bello & Martinez-Monzó (2007) reported that increased of *a** in meat is due both to denaturation of deoxymyoglobin and oxymioglobin and to increase in concentration of metmyoglobin and sulfomyoglobin.

Fischer et al. (2013) stated that phenolic compounds degrade by various extrinsic factors, such as presence of certain compounds and their degradation in the food matrix, and that the loss of anthocyanins is reflected on increased lightness, decreased *a**, but constant or increased *b**

values. Radocaj et al. (2014) reported that RB contains more polyunsaturated fatty acids than BB, and that the total tocopherol content is higher in BB than in RB.

Almeida et al. (2015) added 1.0% aqueous extract of jabuticaba to the elaboration of Bologna-type mortadella, evaluating the stability of color characteristics during 35-day storage under refrigeration at 4°C. The authors reported a statistical difference in lightness during storage, with decrease in *L** value, from 65.6 to 63.3; however, there was no difference of *a** value during storage.

3.2.3 Total phenolic content and antioxidant capacity

All cooked chicken burger treatments had a significant difference in relation to total phenolic content. F1 and F2 presented the highest values (4.59 and 3.43 mg GAE/g of chicken burger on a dry basis, respectively), when compared with CF1 and CF2 (2.24 and 1.78 mg GAE/g of chicken burger on a dry basis, respectively). The values obtained for F1 and F2 are similar to those reported by Alves et al. (2017) for restructured hams added with 1.5% jabuticaba peel flour, and was 2.50 mg GAE/g of restructured hams on a dry basis.

There was difference between F1 and F2 regarding the antioxidant capacity verified by the ABTS assay, where F1 presented 93.9 μmol of Trolox equivalents/g of chicken burger on a dry basis, and F2 showed 80.87 μmol of Trolox equivalents/g of chicken burger on a dry basis. CF1 and CF2 presented the lowest values of antioxidant capacity, with 71.37 and 64.83 mg of Trolox equivalents/g of chicken burger on a dry basis, respectively.

Determination of total phenolic content in chicken burgers with addition RB and BB was very important to verify whether phenolic compounds degraded over storage time and during chicken burger cooking. Total phenolic content and antioxidant capacity between RB and cooked chicken burger with addition of RB decreased by 82.85% and 86.57%, respectively, and between BB and chicken burger with addition of BB decreased by 89.62% and 89.65%, respectively. On the

other hands, on average, the total phenolic content and antioxidant activity between control treatments (CF1 and CF2) and treatments with addition of by-products (F1 and F2) increased by 99,50% and 28,31%.

Despite the drastic decrease in phenolic compound concentration, chicken burgers with addition of RB and BB kept on presenting phenolic compounds that will remain available in the meat matrix. It improves its functional characteristics and makes the reformulated chicken burgers healthier for not containing added antioxidants, and for the very health benefits of phenolic compounds (Biswas et al., 2011; Cetojevic-Simin et al., 2015; Salaheen et al. 2014).

Babbar et al. (2011) reported that certain food constituents, such as ascorbates, carbohydrates, tocopherols, carotenoids, terpenes, and some other dyes, might have a synergistic effect on each other and contribute to total antioxidant activity, there being a synergistic or antagonistic effect between phenolic and non-phenolic compounds.

3.2.4 Cooking characteristics

Table 5 shows the percentages of weight loss on cooking and diameter shrinkage in chicken burger samples, evaluated for 60 days.

It can be noted that there was no difference in the percentages of cooking loss and diameter shrinkage during 60-day storage and between treatments. One explanation for this fact is that dietary fibers have the properties of water holding capacity decreasing cooking loss and diameter shrinkage, however, depends on the types of fibers, its concentration, temperature, pH and presence of ions (Biswas et al., 2011). Another ingredient that helps in water holding capacity is maltodextrin, avoiding cooking losses and diameter shrinkage (Feiner, 2006).

Table 5. Percentage of cooking loss (% CL), percentage of diameter shrinkage (% DS) and texture profile of chicken burgers throughout storage time.

Measurement	Storage time (days)	CF1	CF2	F1	F2
% CL	0	31,17±4,86^{aA}	39.11±2.31^{aA}	32.70±2.44^{aA}	37.14±1.45^{aA}
	15	34,56±3,70^{aA}	36.69±1.56^{aA}	32.88±1.55^{aA}	34.65±2.45^{aA}
	30	35,99±1,70^{aA}	38.17±1.74^{aA}	34.05±2.74^{aA}	33.25±2.79^{aA}
	45	35,11±0,72^{aA}	36.79±2.02^{aA}	31.41±4.62^{aA}	35.31±1.93^{aA}
	60	37,42±1,08^{aA}	39.55±1.52^{aA}	33.85±2.94^{aA}	33.84±6.52^{aA}
% DS	0	15.75±1.41^{bA}	22.65±2.83^{aA}	16.12±2.41^{bA}	17.56±1.88^{abA}
	15	20,72±3,47^{aA}	18.23±2.90^{aA}	17.74±3.44^{aA}	18.10±0.99^{aA}
	30	18,91±0,20^{aA}	20.53±2.80^{aA}	17.10±5.17^{aA}	16.74±0.59^{aA}
	45	20,29±1,05^{aA}	19.33±1.47^{aA}	17.83±2.32^{aA}	18.76±3.48^{aA}
	60	19,24±6,85^{aA}	24.58±3.27^{aA}	15.69±2.62^{aA}	19.14±4.64^{aA}
Hardness (N)	0	159.72±42.76^{bC}	179.88±28.49^{abAB}	203.56±41.90^{aAB}	205.42±39.05^{aA}
	15	176.13±41.41^{aBC}	167.55±33.37^{aB}	190.5±29.55^{aBC}	174.59±24.62^{aB}
	30	187.57±40.02^{bB}	190.9±25.96^{bA}	227.74±31.26^{aA}	195.47±28.43^{bAB}
	45	217,9±22,58^{aA}	199.43±26.51^{abA}	188.77±40.91^{bBC}	209.11±25.26^{abA}
	60	178.21±18.57^{abBC}	189.25±27.79^{aAB}	168.54±28.61^{bC}	180.2±30.34^{abB}
Elasticity	0	0.61±0.05^{abB}	0.63±0.06^{aA}	0.62±0.07^{abAB}	0.57±0.07^{bAB}
	15	0.62±0.11^{aB}	0.57±0.08^{abB}	0.6±0.07^{aB}	0.54±0.05^{bB}
	30	0.61±0.10^{abB}	0.64±0.06^{aA}	0.65±0.05^{aA}	0.58±0.05^{bAB}
	45	0,71±0,05^{aA}	0.65±0.05^{bA}	0.61±0.06^{bcAB}	0.6±0.06^{cA}
	60	0.66±0.05^{aAB}	0.64±0.07^{aA}	0.59±0.06^{bB}	0.56±0.06^{bAB}
Cohesiveness	0	0.46±0.06^{aB}	0.46±0.04^{aA}	0.49±0.05^{aAB}	0.47±0.03^{aA}
	15	0,52±0,07^{aA}	0.47±0.05^{bcA}	0.50±0.04^{abA}	0.43±0.04^{cBC}
	30	0,54±0,05^{aA}	0.48±0.04^{bA}	0.49±0.05^{bA}	0.43±0.03^{cB}
	45	0,52±0,04^{aA}	0.48±0.04^{bA}	0.46±0.04^{bcBC}	0.44±0.02^{cB}
	60	0.47±0.2^{aB}	0.47±0.02^{aA}	0.44±0.03^{bC}	0.41±0.02^{cC}
Gumminess	0	47.43±22.45^{aB}	59.33±33.11^{aAB}	63.49±21.25^{aAB}	57.38±18.06^{aA}
	15	59.85±27.97^{aB}	46.1±15.85^{abB}	58.4±16.27^{aBC}	41.62±11.26^{bB}
	30	63.54±23.55^{abB}	59.36±14.19^{bAB}	74.95±21.54^{aA}	49.98±14.01^{bAB}
	45	80,91±15,18^{aA}	63.46±15.40^{bA}	55.17±21.48^{bBC}	55.79±11.67^{bA}
	60	55.23±9.44^{aB}	57.87±13.56^{aAB}	44.41±11.18^{bC}	42.12±11.69^{bB}

Equal horizontal lower cases indicate no difference between the samples, and vertical capital letters indicate no difference regarding storage time. N, newton. The table shows the values of means ± standard deviation.

On average, from the highest to the lowest, the percentage of cooked loss was 38.06% > 34.85% > 34.84% > 32.98% (CF2 > CF1 > F2 > F1), and the percentage of diameter shrinkage was 21.06% > 18.98% > 17.46% > 16.90% (CF2 > CF1 > F2 > F1). Cooked chicken burger treatments with addition of RB and BB lost less weight and diameter.

Selani et al. (2016) studied the effect of addition of pineapple processing by-product on the elaboration of bovine burger. The burger formulation consisted of beef, bacon, water, canola oil emulsion, pineapple by-product (bark and bagasse), salt, maltodextrin, sodium polyphosphate, sodium erythorbate, spices and monosodium glutamate. There was addition of 1.5 g of by-product/100g of burger. In addition, reported cooking properties of 39.92% of cooking loss and 20.45% diameter shrinkage.

3.2.5 Texture profile

Table 5 presents the results of the evaluation of chicken burger texture profile with and without the addition of fruit by-products, monitored for 60 days.

Ziegler, Rizvi & Acton (1987) reported that the hardness is inversely proportional to the moisture content of the meat product. The treatments F1 and F2, presented greater hardness when compared to the treatments CF1 and CF2 in sample right after processing (0-day time), due to the fact that they presented lower moisture content when compared with the treatments.

CF1 and CF2 maintained the degree of hardness, elasticity, cohesiveness and gumminess, and F1 and F2, in turn, showed a significant decrease in hardness, cohesiveness and gumminess values during 60-day storage. Therefore, the addition of RB and BB decreased the hardness, elasticity, cohesiveness and gumminess of the samples during storage time. Sánchez-Zapata et al. (2010) concluded that the addition of by-product of tiger nut in the elaboration of pork burger decreases the hardness, elasticity, cohesiveness and gumminess.

In general, addition of raspberry and blackberry pulp processing by-products caused improvements in texture profile in chicken burgers stored during the 60-day time.

4. Conclusion

Raspberry and blackberry pulp processing by-products presented nutritional, dietary and functional characteristics for their application to substitute food additives. Addition of raspberry and blackberry by-products in chicken burger changed color, and increased lipid content and phenolic compounds becoming functional food, inhibiting or retarding lipid and protein oxidation, and improving cooking characteristics and texture profile.

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CHAPTER 3

Effect of the application of raspberry (*Rubus idaeus*) and blackberry (*Rubus brasiliensis*) pulp processing by-products on lipid oxidation and sensory characteristics of chicken burger

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Effect of the application of raspberry (*Rubus idaeus*) and blackberry (*Rubus brasiliensis*) pulp processing by-products on lipid oxidation and sensory characteristics of chicken burger

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ABSTRACT

The berries pulp industry generates by-products that are little used, although they feature significant amounts of antioxidant compounds. In this context, the objective of this work was to evaluate the effect of the application of by-products of pulp processing of raspberry (RB) and blackberry (BB) on oxidation stability in eight formulations of chicken burger. Being four formulations with mechanically separated chicken meat (MSCM), and four without MSCM during storage time, besides, to assess sensory characteristics in chicken burger with RB and BB during 60 days of storage. TBARS values (mg MDA/kg) in samples with and without MSCM and acceptance testing and the CATA (check-all-that-apply) test were determined. Treatments with addition of RB and BB had an antioxidant effect, and overcame the antioxidant effect of sodium ascorbate. Acceptance of the treatments with RB and BB was moderate. However, adjustments are still required in the formulation.

Keywords: Chicken burger, MSCM, fruit by-product, lipid oxidation, natural antioxidant, sensory stability.

1. Introduction

Burger is one of the meat products of most consumption worldwide and with a wide variety of formulations depending on the type of meat, production cost, and nutritional value (Feiner, 2006). Burger processing conditions, such as grinding and salt addition, freezing rate,

storage conditions and time, cooking parameters, among others, accelerate oxidation reactions in the meat matrix (Gheisari, & Motamedi, 2010; Soyer, Özalp, Dalmış, & Bilgin, 2010).

Chicken meat is one of the meats with greater acceptance worldwide, and remains gaining in popularity due to its competitive price. However, chicken meat is more susceptible to oxidation because of the larger quantity of phospholipids compared to other types of meat, which in turn, with presence of unsaturated fatty acids (Wilson, Pearson, & Shorland, 1976). Mechanically separated chicken meat (MSCM) is obtained from crushing the tissues after removing the meat from the chicken carcass. It mainly consists of cartilage, skin, leftovers of flesh and bones, in addition to being rich in minerals and fats, but with low protein content. MSCM is highly susceptible to lipid oxidation for being exposed to oxygen during mechanical deboning and for being rich in heminic iron (Negrão et al., 2005).

Freezing is a conservation method that prolongs the shelf life of meat products, ensuring food safety during transportation and commercialization. However, the temperature at which the product is frozen and stored determines the amount of water available for oxidation reactions to happen, and which will accelerate lipid oxidation during defrosting (Ali, Zhang, Rajput, Khan, Li & Zhou, 2015; Utrera, Morcuende & Estévez, 2014). The use of high temperatures during cooking destroys the cellular structure of the meat and meat products, allowing interaction between polyunsaturated fatty acids and prooxidants, such as iron, thus beginning the oxidation processes (Sato, & Hegarty, 1971).

The addition of salt (sodium chloride) in a standard burger formulation varies between 0.2 and 1.5%. Salt helps in the extraction of myofibrillar proteins during the preparation of meat products, besides contributing to the taste of the product. Nevertheless, addition above 0.9% of salt promotes lipid oxidation in raw or cooked meat, and accelerates the formation of metmyoglobin and the discoloration of raw meats, because salt is a prooxidant (Feiner, 2006; Rhee & Ziprin, 2001).

Lipid oxidation is one of the major causes of deterioration in the quality of meat and meat product stored, affecting sensory characteristics (Ladikos, & Lougovois, 1990). However, the use of antioxidants is deemed necessary to inhibit or delay lipid oxidation and the shelf life of meat products. Synthetic antioxidants commonly used by the food industry are the butylhydroxyanisole (BHA), butylhydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ). On the other hand, some authors suggest that these food additives may be harmful to health due to their relation with toxicological effects (Sarafian, Kouyoumjian, Tashkin, & Roth, 2002). Another way, still little explored, is the use of antioxidants obtained from the extraction of phenolic compounds, present in fruits and their by-products (Sah, Don Bosco, & Mir, 2014).

Berries are small fruits widely cultivated throughout the world, which have a high content of bioactive compounds such as phenolic and ascorbic acid, among others (Kalt, Forney, Martin, & Prior, 1999; Souza, Pereira, Silva, Lima, Pio & Queiroz, 2014). Processing of pulp and juice berries generates a considerable amount of by-products consisting of peel, seed, and fruit leftovers that, like the entire berries, also have bioactive compounds with functional properties (Gouw, Jung, & Zhao, 2017; Alves, Marques, Carvalho, Pinheiro, Ramos & Corrêa, 2017). And, when added to food can bring important health benefits due to their antioxidant, anti-microbial, anti-inflammatory, anticancer, antihypertensive, and cardioprotective properties (Bowen-Forbes, Zhang, & Nair, 2010; Liobikas, Skemiene, Trumbeckaite, & Borutaite, 2016).

There is a growing need for healthier or Clean Label meat products, substituting food additives for ingredients, which have no side effects on health and add nutritional value to the food (Gouw, Jung, & Zhao, 2017). Such demand makes the use of by-products of berries an alternative to obtain healthier ingredients for use in functional food production, in addition to benefiting the consumers' health (Kowalska, Czajkowska, Cichowska, & Lenart, 2017).

Therefore, the objective of this work was to evaluate the effect of the application of raspberry (*Rubus idaeus*) and blackberry (*Rubus brasiliensis*) pulp processing by-products on oxidation and sensory stability in chicken burger during storage time.

2. Material and methods

2.1 Obtaining and packaging of raw materials

Raspberry (*Rubus idaeus*) and blackberry (*Rubus brasiliensis*) pulp processing by-products, consisting of peel and seed, were obtained from the Sítio do Bello producer, located in the city of Paraibuna, São Paulo (S 23º 23', W 45º 39') in November 2016. Soon after obtaining by-products, they were packed in plastic bags with protection from light, frozen, and then transported to the Laboratoty of Fruit, Vegetable and Confectionery Products of the State University of Campinas, where remained frozen at -18 °C until drying. The by-products were dried by convection in air circulation oven (Marconi MA035, Brazil) at 40 °C for 48 hours, and ground in a Grindomix GM200 Knife Mill (Restch, Germany) until obtainment of ≤ 0.21 mm particles (65 Mesh). Then, by-products were stored at -18 °C in vacuum metallized polyethylene bags until chicken burger were made.

Refrigerated chicken meat, particularly chicken breast fillet, was obtained in a commercial establishment in Campinas-SP. The meat was transported in plastic bags to the Laboratory of Meat and Meat Products of the State University of Campinas, and maintained under refrigeration. Meat pH was measured to verify the good condition of the raw material; then it was manually chopped and ground using a 5-mm grinding disc meat chopper (CAF model 22STB, Brazil), and maintained at -2 ºC until the next day, when the chicken burgers were made. Mechanically separated chicken meat (MSCM) was obtained from slaughterhouse in Holambra-SP, and kept frozen at -18 ºC in vacuum plastic package.

2.2 Treatments

After preliminary tests to determine the percentage of addition of raspberry and blackberry by-products in chicken burger, eight formulations of chicken burger were prepared: four control formulations and four others with addition of raspberry and blackberry pulp processing by-products (Table 1).

Table 1. Formulations of chicken burgers with and without addition of mechanically separated chicken meat and raspberry and blackberry by-products.

Ingredients	**%**							
	CF1	**CF2**	**CF3**	**CF4**	**F1**	**F2**	**F3**	**F4**
Chicken meat	73	73	53	53	73	73	53	53
MSCM*	-	-	20	20	-	-	20	20
Canola oil	5	5	5	5	5	5	5	5
Water	18.15	18.2	18.15	18.2	16.2	16.2	16.2	16.2
Raspberry by-products	-	-	-	-	2	-	2	-
Blackberry by-products	-	-	-	-	-	2	-	2
Sodium chloride	2	2	2	2	2	2	2	2
Sodium ascorbate	0.05	-	0.05	0	-	-	-	-
Maltodextrin	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Total	100	100	100	100	100	100	100	100

* Mechanically separated chicken meat

2.3 Cooked chicken burger processing

Chicken burgers were prepared at the pilot plant of the Laboratory of Meat Science and Technology of the School of Food Engineering, State University of Campinas, in refrigerated environment and at a temperature lower or equal to 10 °C. The preparation was carried out in batches of 3 kg in homogenizer (Filizola-Sire, Brazil). The process was performed in triplicate.

For CF1 and CF2, ground chicken meat, sodium chloride, and water for 60 s; then maltodextrin was added, and the mixture was homogenized for more 60s. Canola oil was added and mixture for additional 20 s. In the case of CF3 and CF4 formulations, ground MSCM was added, in addition to chicken meat, sodium chloride, and water. In the case of CF1 and CF3 formulations, sodium ascorbate was added after maltodextrin and before canola oil, and the mixture was homogenized for more 20 s.

For F1 and F3 formulations, RB powder was added in the first stage of preparation, along with ground chicken meat, sodium chloride, and water. For F2 and F4 formulations, BB powder was added along with ground chicken meat, sodium chloride, and water. For F3 and F4 formulations, MSCM was added in the first stage of preparation, along with ground chicken meat, sodium chloride, water, and the fruit by-product. Then, maltodextrin and canola oil were added. In all the formulations, we noted the time of homogenization for each ingredient, as explained to control formulations.

Finished formulations were stored between 0–1 °C for about one hour, and then chicken burgers of 100 ± 5 g and 100 mm in diameter were made using a burger maker (Müller, Brazil). The chicken burgers were put in low density polyethylene bags, proper for burger, and frozen at -18 °C for about 8 h to take shape.

Afterwards, raw treatments of CF1, CF2, F1 and F2 (burgers with 73% of chicken meat) were stored in vacuum transparent polyethylene bags at -18 °C for up to 60 days for performance of lifespan studies at 0, 15, 30, 45, and 60 storage days. Treatments CF3, CF4, F3, and F4 (chicken burgers with 53% of chicken meat in addition to 20% MSCM) were cooked in an electric plate (Sire, Brazil) at 175 °C for 7 minutes, until reaching 75 °C internal temperature. After cooking, samples were cooled at 25 °C approximately, and packaged in transparent polyethylene bags

without vacuum and stored at 4 °C BOD for 5 days for the completion of the studies on shelf life considering 0, 1, 3, and 5 days.

After reaching the storage time, raw chicken burgers of CF1, CF2, F1, and F2 treatments were cooked in an electric plate (Sire, Brazil) at 175 °C for 7 minutes, until reaching 75 °C internal temperature; then, samples were cooled at 25 °C for the respective analyses.

2.4 Analytical determinations

2.4.1 Lipid oxidation

Lipid oxidation was measured by determining the values of thiobarbituric acid reactive substances (TBARS) of all treatments for each analysis time, following the methodology by Raharjo, Sofos, and Schmidt (1992) with some modifications. An amount of 2.5 g of the sample was homogenized with 10 mL of trichloroacetic acid solution (5%) and 0.25 mL of BHT ethanolic solution (0.19 M) in Ultra-Turrax® digital IKA T-25 at 20000 rpm for 1 min, in ice bath. The sample was centrifuged at 3000 rpm for 10 min, and the supernatant was took. An aliquot of 3 mL of the filter was mixed with 3 mL of thiobarbituric acid solution (0.02 M). The mixture was agitated in vortex and heated in water bath at 100 °C for 60 min. After cooling in ice bath, absorbance at 532 nm was measured by the Orion AquaMate 8000 Uv-Vis spectrophotometer (Thermo Scientific, USA). The values of absorbance of the TEP standard curve at different concentrations (from 0.17 to 1.015 mg TEP/mL) was determined. Analysis was performed in four times, and the result was expressed in mg malonaldehyde/kg sample.

2.4.2 Sensory analysis

Two sensory tests with consumers were conducted: Acceptance testing and Check-all-that-apply – CATA test, for CF1, CF2, F1, and F2 treatments at 0 and 60 storage days. The tests were previously approved by the Ethics Committee of the State University of Campinas, protocol

no. 76442317.1.0000.5404. On the day of the analysis, raw chicken burgers were cooked as explained in item 2.3. Sensory analyses were carried out in individual booths illuminated with white light, at room temperature. Among the samples, evaluators were provided with water at room temperature and salt biscuits for cleansing the palate. Samples, still hot, were randomly presented, one at a time, with the evaluation form. 123 evaluators participated in the sensory tests at 0-day time, and 124 evaluators, at 60-day time. Evaluators consisted in chicken burger consumers aged between 18 and 60 years.

2.4.2.1 Acceptance test

For acceptance test, evaluators responded questions concerning the attributes of appearance, aroma, flavor, texture, and overall impression, using the 9-point structured hedonic scale (from 1, dislike extremely, to 9, like extremely).

2.4.2.2 CATA (check-all-that-apply)

According to the methodology described by Ares et al. (2013) and Jaeger et al. (2015), the attributes evaluated in the CATA test for each storage time were previously selected with the aid of 15 untrained evaluators, chicken burger consumers. For the survey of attributes, evaluators received four samples of chicken burger, presented in pairs, and were asked to evaluate similarities and differences between them. Results of the study were calculated by the frequency (%) in which attributes were cited, being 26 terms set to the 0-day time, and 28 terms to the 60-day time to characterize the chicken burger samples, as described in Table 2. The evaluators identified, through markings in the forms, which presented terms characterized each sample the best. The terms were randomly sorted to avoid possible marking vices.

Table 2. Attributes determined for CATA (check-all-that-apply) analysis for times 0 and 60 days of storage of chicken burger

Sensory analysis	CATA attributes
1st Sensory analysis (0-day time): Chicken burger right after processing	- Appearance: beef burger, moist - Color: yellow, brown, pink, purple - Aroma: barbecue, chicken, fruit, rancid, spicy - Taste: bitter, sweet, salty - Flavor: barbecue, chicken, fruit, spicy, strange, metallic, rancid, residual, dry - Texture: juicy, firm, soft
2nd Sensory analysis (60-day time): Chicken burger with 60 days of storage	- Appearance: beef burger, moist, dry* - Color: yellow, white*, brown, pink, purple - Aroma: barbecue, chicken, fruit, rancid, spicy - Taste: bitter, sweet, salty - Flavor: barbecue, chicken, fruit, spicy, strange, metallic, rancid, residual, dry - Texture: juicy, firm, soft

* Attributes included in the 2nd sensory analysis for CATA (check-all-that-apply) evaluation.

2.5 Statistical analysis

Results of the determination of lipid oxidation were analyzed using analysis of variance, with the Generalized Linear Model (GLM), and means compared by Tukey test, considering a 5% significance level ($P<0.05$). Statistical analyses were performed using the Statistica software package (v7.0, StatSoft, Inc., USA, 2004).

Data on the sensory analysis for samples right after processing (0-day time) and for those with 60 days of storage (60-day time) were analyzed using the XLSTAT (Addinsoft, Paris, France) program, using analysis of variance (ANOVA), and means compared by Tukey test considering a 5% significance level ($P<0.05$). CATA data were analyzed by Cochran's Q test,

comparing the samples with each attribute independently, and Multiple Factor Analysis (MFA) related these data among treatments.

3. Results and discussion

3.1 Lipid oxidation

In this study, it was used 2% salt (sodium chloride) in all treatments of chicken burger, aiming at accelerating oxidation reactions and verifying the antioxidant effect of the addition of RB and BB in chicken burgers. Treatments of chicken burgers were assessed regarding lipid oxidation by the TBARS index, and the results are presented in Table 3.

Table 3. TBARS values (mg MDA/kg sample) of chicken burgers with or without addition of raspberry and blackberry pulp processing by-products, with or without mechanically separated chicken meat (MSCM) during storage time

Time (days)	Without MSCM*			
	CF1	CF2	F1	F2
0	0.21±0.01^{dC}	0.25±0.01^{cC}	0.32±0.03^{bB}	0.43±0.01^{aA}
15	0.26±0.0^{cB}	0.49±0.02^{aA}	0.30±0.01^{bB}	0.32±0.01^{bC}
30	0.29±0.01^{cAB}	0.43±0.01^{aB}	0.33±0.02^{bAB}	0.32±0.01^{bC}
45	0.27±0.01^{cB}	0.48±0.01^{aA}	0.38±0.02^{bA}	0.38±0.02^{bB}
60	0.31±0.03^{bA}	0.48±0.03^{aA}	0.34±0.02^{bAB}	0.34±0.01^{bC}
	With MSCM*			
	CF3	CF4	F3	F4
0	0.45±0.02^{bB}	0.54±0.01^{aC}	0.41±0.01^{cA}	0.48±0.01^{bA}
1	0.40±0.02^{cC}	0.49±0.05^{aC}	0.48±0.01^{abA}	0.41±0.01^{bcB}
3	0.48±0.02^{bAB}	0.69±0.01^{aB}	0.41±0.03^{cA}	0.39±0.02^{cB}
5	0.50±0.01^{bA}	0.89±0.02^{aA}	0.42±0.05^{bcA}	0.41±0.04^{cB}

* MSCM, mechanically separated chicken meat.

- CF1 and CF3, control with sodium ascorbate; CF2 and CF4, control without adding antioxidant; F1 and F3, with addition of raspberry by-products; F2 and F4, with addition of blackberry by-products.

- Equal lowercase letters on the same line indicate there was no significant difference ($p<0.05$) between means by the Tukey test for the same storage time. Equal uppercase letters on the same column indicate there was no significant difference ($p<0.05$) between means by the Tukey test for the same treatment in different storage times.
- The table shows the values of mean ± standard deviation.

Among treatments without MSCM (CF1, CF2, F1, and F2), F1 (with addition of RB) and F2 (with addition of BB) treatments featured higher TBARS values than treatment CF1 (with addition of sodium ascorbate) during the first weeks of the study. A possible justification for such fact is that with the grinding, the polyunsaturated fatty acids present in the seed of RB and BB were exposed, stimulating the oxidation (Radocaj et al., 2014), making F1 and F2 treatments more susceptible to lipid oxidation soon after processing (0-day time) (Soyer et al., 2010). However, it is noteworthy that in F1 and F2 treatments there was stability in TBARS values concerning the storage time, whereas for CF1 and CF2 there was an increase of values. After 60 days of storage, TBARS values for CF1, F1, and F2 treatments did not differ among themselves, demonstrating that RB and BB have the same antioxidant effectiveness as sodium ascorbate.

On the other hand, for the treatment with MSCM (CF3, CF4, F3, and F4), TBARS values on the fifth day of storage for F3 (with addition of RB) and F4 (with addition of BB) were the lowest when compared with the other treatments with MSCM, however, F4 treatment decreased of TBARS values over the storage time. A possible explanation is the higher presence of phenolic compounds and total tocopherols in BB, than RB (Radocaj et al., 2014), which increases the antioxidant power of BB when compared with RB (Kowalska et al., 2017). For CF4 treatment (with no addition of antioxidant) featured the highest TBARS values during the 5 days of storage. After 5 days of storage, CF3 and F3 treatments, and F3 and F4 treatments did no differ from each other, demonstrating that RB and BB have the same antioxidant effectiveness as sodium ascorbate.

Overall, there was a significant increase of lipid oxidation over storage time for control treatments (CF1, CF2, CF3, and CF4), which was not observed in treatments with addition of RB

(F1 and F3), whose values remained constant; and treatments with addition of BB (F2 and F4), whose values presented reduction over storage time.

RB and BB contains phenolic compounds and antioxidant activity (Kalt et al., 1999; Souza et al., 2014). As reported by Jacinto-Valderrama et al., (no press) F1 and F2 treatments presented the highest values in total phenolic content when compared with CF1 and CF2 treatments. To complement the study, some data not yet published are that the total phenolic content in CF3 and CF4 treatments was 1.37 and 1.07 mg gallic acid/g of chicken burger on dry basis, respectively, and in F3 and F4 treatments was 3.11 and 2.24 mg gallic acid/g of chicken burger on dry basis, respectively. Therefore, treatments F1, F2, F3 and F4 presented the highest values in total phenolic content when compared to control treatments CF1, CF2, CF3 and CF4. Besides, the antioxidant activity in CF3 and CF4 treatments was 47.33 and 48.06 µmol of Trolox equivalents/g of chicken burger on dry basis, respectively, and in F3 and F4 treatments was 65.62 and 45.01 µmol of Trolox equivalents/g of chicken burger on dry basis, respectively. These data demonstrate that F3 and F4 treatments present the highest values in total phenolic compounds and antioxidant activity when compared with CF3 and CF4 treatments.

Treatments with RB and BB addition, the total phenolic content in the F3 and F4 treatments was lower than in the F1 and F2 treatments. An explanation for this fact is that MSCM present in F3 and F4 treatments stimulated the loss of phenolic compounds, because it is highly susceptible to lipid oxidation (Negrão et al., 2005). Moreover, since the F1 and F2 treatments had no MSCM in their composition, there was less loss of phenolics. Another fact to consider is that the F1 and F2 treatments were stored raw in vacuum polyethylene bags for 60 days at -18 ° C, however, the treatments F3 and F4 were stored cooked in polyethylene bags without vacuum for 5 days at 4 ° C. Therefore, treatments F3 and F4 were exposed to oxygen during storage days.

A study conducted by Naavena, Sem, Vaithiyanathan, Babji, & Kondaiah (2008) on chicken burger with addition of pomegranate juice, pomegranate rind powder extract (60 mesh), and BHT. Samples were cooked in microwave oven until reaching 80 °C of internal temperature, and then stored for 15 days under refrigeration at 4° C. They concluded that adding pomegranate rind powder extract had greater effectiveness in reducing lipid oxidation over the 15 days of storage when compared with the other treatments. Moreover, on the 15th day of storage, TBARS values accounted for 1.272 mg MDA/kg sample for the control burger; 0.763 mg MDA/kg sample for the burger with addition of pomegranate juice; 0.203 mg MDA/kg sample for the burger with addition of pomegranate rind powder extract; and 0.896 mg MDA/kg sample for the burger with addition of BHT.

Sáyago-Ayerdi, Brenes, and Goñi (2009) added 1 and 2% of powdered by-product (0.5 mm) of the processing of grape wine in chicken burgers, cooked at 170 °C for 3 min, and stored for 5 days under refrigeration, and reported that there was no significant difference in TBARS values on the fifth day of storage, accounting for 1.59 mg MDA/kg sample for burgers with 1% grape by-product; 1.35 mg MDA/kg sample for burger with 2% grape by-product; and 1.82 mg/kg sample for the control burger.

3.2 Sensory analysis

3.2.1 Acceptance testing

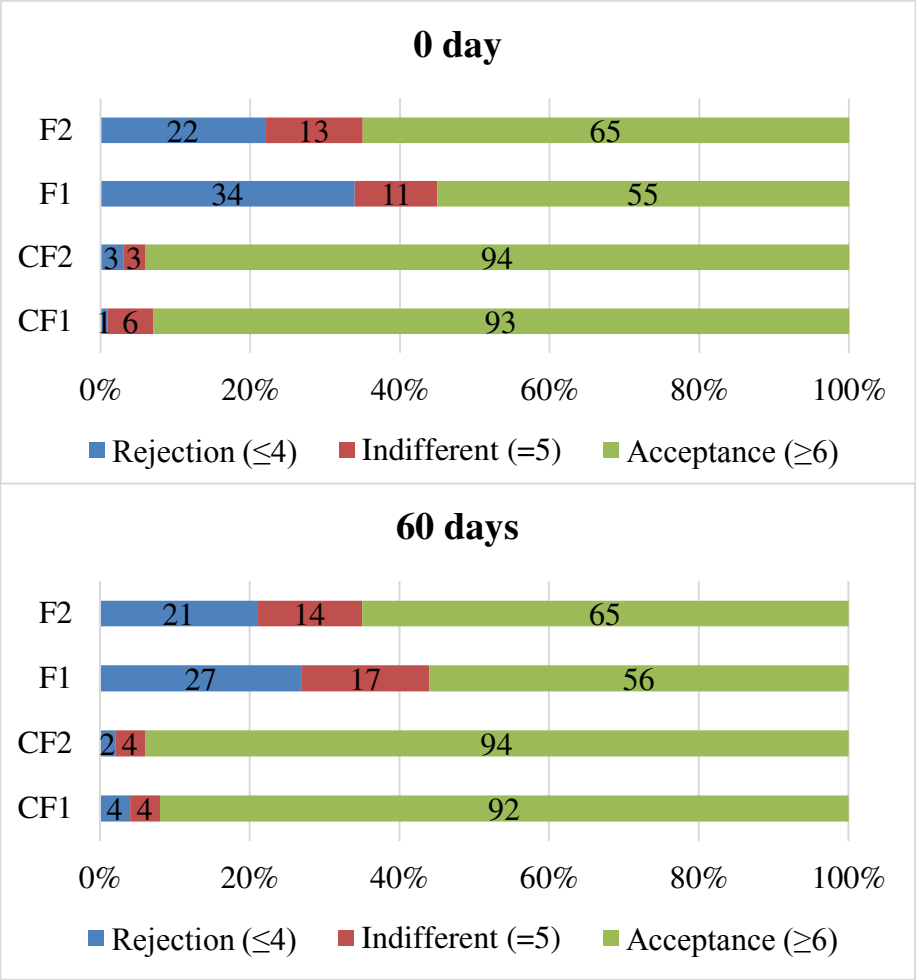
The averages for attributes of appearance, aroma, flavor, texture, and overall impression of chicken burgers with 0 and 60 days of storage are found in Table 4, and the acceptance percentages, in Figure 1.

Table 4. The averages for attributes of appearance, aroma, flavor and texture, and overall impression of chicken burgers with 0 and 60 days of storage

	Time (days)	CF1	CF2	F1	F2
Appearance	0	6.9±1.6^{a}	7.1±1.5^{a}	5.11±2.1^{b}	5.7±2.0^{c}
	60	6.6±1.7^{a}	7.1±1.5^{a}	6.5±1.6^{a}	5.9±2.0^{b}
Aroma	0	7.0±1.4^{a}	7.0±1.3^{a}	5.8±1.8^{b}	6.6±1.4^{a}
	60	6.6±1.5^{a}	6.8±1.5^{a}	6.3±1.6^{a}	6.6±1.6^{a}
Flavor	0	7.5±1.3^{a}	7.5±1.4^{a}	5.1±2.2^{c}	5.8±2.0^{b}
	60	7.4±1.3^{a}	7.4±1.4^{a}	5.3±2.2^{b}	5.9±2.1^{b}
Texture	0	7.4±1.4^{a}	7.3±1.5^{a}	6.2±1.7^{b}	6.5±1.8^{b}
	60	7.3±1.4^{a}	7.4±1.1^{a}	6.0±1.7^{c}	6.7±1.4^{b}
Overall Impression	0	7.4±1.2^{a}	7.3±1.3^{a}	5.3±1.9^{c}	5.9±1.8^{b}
	60	7.2±1.3^{a}	7.3±1.2^{a}	5.8±1.9^{b}	6.1±1.8^{b}

- CF1, control with sodium ascorbate; CF2, control without adding antioxidant; F1, with addition of raspberry by-products; F2, with addition of blackberry by-products.
- 0-day and 60-days time: equal lowercase letters on the same line indicate no significant difference ($p<0.05$) between the samples by the Tukey test for the same sensory attribute.

Overall, the control samples (CF1 and CF2) achieved higher acceptance scores in all attributes when compared with other treatments in both times in which the analysis was performed, evaluated between “like slightly” and “like moderately”. On the other hand, samples with added RB and BB featured evaluations between “neither like or dislike” and “like slightly”. For acceptance percentages, the control samples (CF1 and CF2) had acceptance between 92 and 94% and, on the contrary, samples with addition of by-products (F1 and F2) had acceptance between 55 and 65%. In addition, between 11 and 17% were indifferent about the acceptance of samples F1 and F2.



- CF1, control with sodium ascorbate; CF2, control without adding antioxidant; F1, with addition of raspberry by-products; F2, with addition blackberry by-products.

Figure 1. Acceptance percentage of chicken burger with 0 and 60 days of storage

On the other hand, when comparing the averages of acceptance for attributes in the samples with 0 and 60 days of storage, it is noteworthy that there was a decrease in such values in all the attributes of the control samples (CF1 and CF2), except in the “texture” attribute of CF2 sample, which has a slight improvement. However, samples of F1 and F2 treatments had an increase in the values of acceptance in all attributes, except in the “texture” attribute of F1 sample, which featured a slight decrease of the mean. Moreover, when comparing the statistical differences among treatments in the samples with 60 days of storage, we observed that samples with added RB (F1) had no difference concerning the “appearance” attribute when compared with control

treatments (CF1 and CF2); samples with added RB and BB had no difference regarding the "aroma" attribute when compared with control treatments (CF1 and CF2).

Moderate acceptance of F1 and F2 treatments on the part of the evaluators is probably related to phenolic compounds and dietary fibers contents in RB and BB, which consequently promoted changes in the following attributes: appearance, aroma, flavor, and texture by modifying the sensory concept of traditional chicken burger. Phenolic compounds give color, bitter taste, and sensation of astringency when added to the food (Dinnella, Recchia, Tuorila, & Monteleone, 2011; Soto-Vaca, Gutierrez, Losso, Xu & Finley, 2012). The bitter taste and sensation of astringency in the mouth are due to the interaction between salivary proteins and phenolic compounds, forming a complex that causes salivary proteins to precipitate in the mouth (Jakobek, 2015).

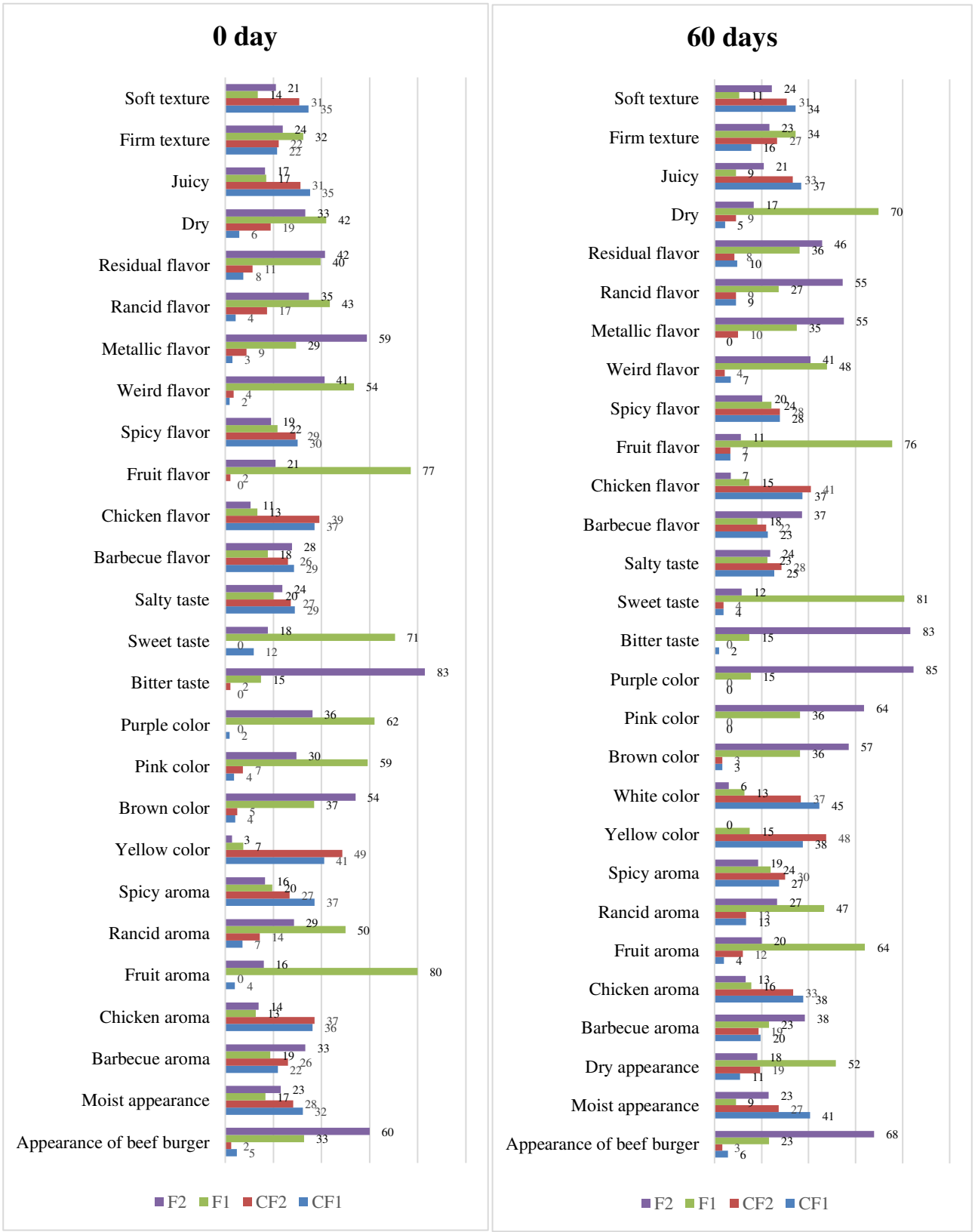
The biggest challenge in the reformulation of meat products with addition of fruits or their by-products is the low acceptance of the characteristics of color, aroma, flavor, and texture, since consumers do not accept very severe changes that may alter the concept of a standard formulation of any meat product. Haugaard, Hansen, Jensen & Grunert (2014) studied consumers' attitude and purchase intent concerning meat products with added herbs and berries. Participants stated that if changes in the color are minimal and of easy adaptation, they could accept the new product, and that the color of the fruit to be used should be related to the color of the meat product. Regarding texture and aroma, few participants stated they do not mind the changes of these attributes; however, other participants expressed they would probably need some time to get used to the changes. Participants mentioned that flavor is the main factor to determine the intention to purchase the reformulated meat product. Moreover, a good dissemination of the health benefits and of the application of berries in the conservation of foods may influence on the intention of purchase, although depending on the price.

Alves at al. (2017) added powdered jabuticaba skin (35 mesh) in the preparation of restructured ham and conducted the acceptance testing, using the 9-point structured hedonic scale, to evaluate the attributes: appearance, aroma, flavor, texture, and overall impression. After the first test, the evaluators were informed that the addition of jabuticaba by-product brings health benefits, and then they were asked to reevaluate the overall impression attribute. Treatment with addition of 1.5% jabuticaba by-product featured the lowest means of rates concerning acceptance among the treatments with addition of such by-product. On the other hand, the authors observed that means of acceptance rates increased as the concentration of jabuticaba by-product decreased.

Several studies have reported that the use of food additives, which are common in the food industry, can lessen the perception of bitter taste and the sensation of astringency in foods. Ares, Barreiro, Delize & Gámbaro (2009) studied the effect of the addition of sucrose, sucralose, polydextrose, and powdered milk on lessening the bitter taste and sensation of astringency in teas of two Uruguayan plants. They concluded that adding 8% sucrose, 3% polydextrose, 0.013% sucralose, and powdered milk with 0% and 3.2% fat to samples of tea had significantly lessened the bitter taste and the sensation of astringency. In another study, conducted by Soares, Mateus & Freitas (2012), the authors analyzed the effect of the addition of acacia gum, pectin, and polygalacturonic acid on the interaction between human saliva and procyanadin extracts of grape seeds. They concluded that pectin (5 g/L water) was the most effective carbohydrate in inhibiting the precipitation of salivary proteins by condensed tannins, followed by acacia gum (10 g/L water), and polygalacturonic acid (20.0 g/L water).

3.2.2 CATA (check-all-that-apply)

In Figure 2 we show the frequency, in percentage, for every attribute mentioned in CATA concerning both storage times (0 and 60 days).



CF1, control with sodium ascorbate; CF2, control without adding antioxidant; F1, with addition of raspberry by-product; F2, with addition of blackberry by-product.

Figure 2. Frequency of attributes in CATA in times 0 and 60 days of storage

The most cited attributes in CATA in samples right after processing (0-day time) for CF1 treatment were: *yellow color, spicy aroma, chicken aroma, chicken flavor, soft texture,* and *juicy*; for CF2 treatment: *chicken aroma, yellow color, chicken flavor,* and *juicy*. For F1 treatment: *rancid aroma, pink color, purple color, strange flavor, residual flavor,* and *rancid flavor*; and for F2 treatment: *appearance of beef burger, brown color, bitter taste, residual flavor,* and *metallic flavor*. For samples with 60 days of storage, the most cited attributes in CATA for CF1 treatment were: *white color, moist appearance, chicken flavor,* and *juicy*; for CF2 treatment: *yellow color, chicken flavor, chicken aroma,* and *juicy*. For F1 treatment: *fruit aroma, rancid aroma, sweet taste, fruit, dry, strange flavor, dry appearance,* and *residual flavor*; and for F2 treatment: *appearance of beef burger, pink, brown, and purple colors, bitter taste, rancid* and *residual flavors*.

In control treatments CF1 and CF2, characteristic attributes of chicken burgers were cited. On the other hand, in F1 and F2 treatments, the evaluators were able to identify changes in the characteristics of flavor and color. Hence, attributes like *bitter taste, residual flavor, metallic, dry,* and *strange flavors* were noted, and these attributes are sensory characteristics of phenolic compounds present in RB and BB (Soto-Vaca et al., 2012). This distinctive perception as for flavor and sensation of astringency in the mouth are due to the interaction between salivary proteins and phenolic compounds, forming a complex that causes salivary proteins to precipitate in the mouth, thus making this sensation of astringency and bitter taste emerge (Jakobek, 2015).

In both times when conducting the CATA test, the *pink* and *purple color* attributes in samples of F1 treatment (with addition of RB), and in the samples of F2 treatment (with addition of BB) was identified *beef burger appearance* and *brown color* attributes. The change in color, in F1 and F2 treatments, is probably due to the presence of anthocyanins in RB and BB, featuring color pigments between pink and purple, and their shades will be associated with pH and the interaction with the compounds of the meat matrix (Jakobek, 2015).

The *brown color* attribute identified in samples of F2 treatment, may be related with the degradation of anthocyanins, which are sensitive to high cooking temperatures, which were present in greater amount in the BB when compared to that RB (Jiménez, Bohuon, Dornier, Bonazzi, Pérez & Vaillant, 2012).

It is noteworthy that significant differences in almost all attributes of the CATA, except *rancid aroma* and *barbecue flavor* attributes, for the samples right after processing (time 0), and *rancid aroma* and *spicy aroma* attributes for samples with 60 days of storage. Although the *rancid aroma* attribute was cited more frequently in samples of the F1 treatment (with addition of RB) on both days of the CATA test, there was no difference in relation to other treatments by Cochran's Q test.

For samples right after processing (0-day time), the highest values in the Cochran's Q test were: for CF1 and CF2 treatments, *chicken aroma, chicken flavor, yellow color, salty taste, juicy,* and *soft texture*; for the F1 treatment: *fruit aroma, brown color, fruit flavor, strange taste,* and *residual flavor*; and for the F2 treatment: *appearance of beef burger, barbecue aroma, brown color,* and *strange flavor*.

For samples with 60 days of storage, the highest values in the Cochran's Q test were: CF1 and CF2 treatments: *chicken aroma, chicken flavor, juicy, soft texture, moist appearance*, and *white color*; for the F1 treatment: *dry appearance, fruit aroma, sweet taste, bitter taste, fruit flavor, strange, dry taste,* and *firm texture*; and for the F2 treatment: *barbecue aroma, brown color, bitter taste, barbecue flavor,* and *residual flavor*.

Overall, by the Cochran's Q test identified the most important characteristics perceived by the evaluators, and the best attributes evaluated in the control samples (CF1 and CF2) were: *chicken aroma*, chicken flavor, soft texture, and *juicy*; however, for F1 and F2 treatments, were: *residual flavor* and *bitter taste*. This information corroborates what was exposed in the frequency

analysis of the CATA test, and as explained above, the attributes: *bitter taste, strange flavor, residual flavor,* and *dry* are sensory attributes from phenolic compounds (Jakobek, 2015).

Through the Multiple Factor Analysis (MFA) it was possible to understand how the treatments are related to each other, helping to identify whether the attributes evaluated in CATA are similar or not (Andrade, Nalério, Giongo, Barcellos, Ares & Deliza, 2018). For samples right after processing (0-day time), F1 and F2 axes of the MFA explained the variability in 98.16% of CATA attributes, considering F1 axis is responsible for 82.81%. For samples with 60 days of storage, with the two dimensions of the MFA it was explained the variability in 97.02%, where F1 axis is responsible for 73.77% (Figure 3 and 4).

Regarding samples right after processing (0-day time), treatments were divided into three groups according to the quadrants where they belong (Figure 3). The first group comprises the control treatments, CF1 and CF2, sharing the same attributes: *spicy aroma, yellow color, chicken flavor, juicy,* and *chicken aroma*. The second group comprises the F2 treatment (with addition of BB), which was characterized by attributes: *bitter taste, appearance of beef burger, metallic flavor, brown color, strange flavor,* and *rancid flavor*. The third group comprises the F1 treatment (with addition of RB), which was characterized by attributes: *purple color, dry, rancid aroma, pink color, fruit flavor, sweet taste, fruit aroma*, and *firm texture*.

Regarding samples with 60 days of storage, treatments were divided into four groups according to the quadrants in which they belong (Figure 4). The first group comprises the F1 treatment (with addition of RB), which was characterized by the attributes: *dry appearance, fruit flavor, firm texture, sweet taste, dry, fruit aroma, rancid aroma,* and *strange aroma*. The second group comprises the F2 treatment (with addition of BB), which was characterized by the attributes: *residual flavor, metallic flavor, brown color, pink color, rancid flavor, appearance of beef burger, barbecue aroma, purple color, bitter flavor,* and *barbecue flavor*. The third group comprises the

CF1 control treatment, which was characterized by attributes: *soft texture, moist appearance, juicy,* and *salty flavor*. And finally, the fourth group comprises the CF2 control treatment, which was characterized by attributes: *chicken aroma, white color, chicken flavor, yellow color, spicy aroma,* and *spicy flavor*.

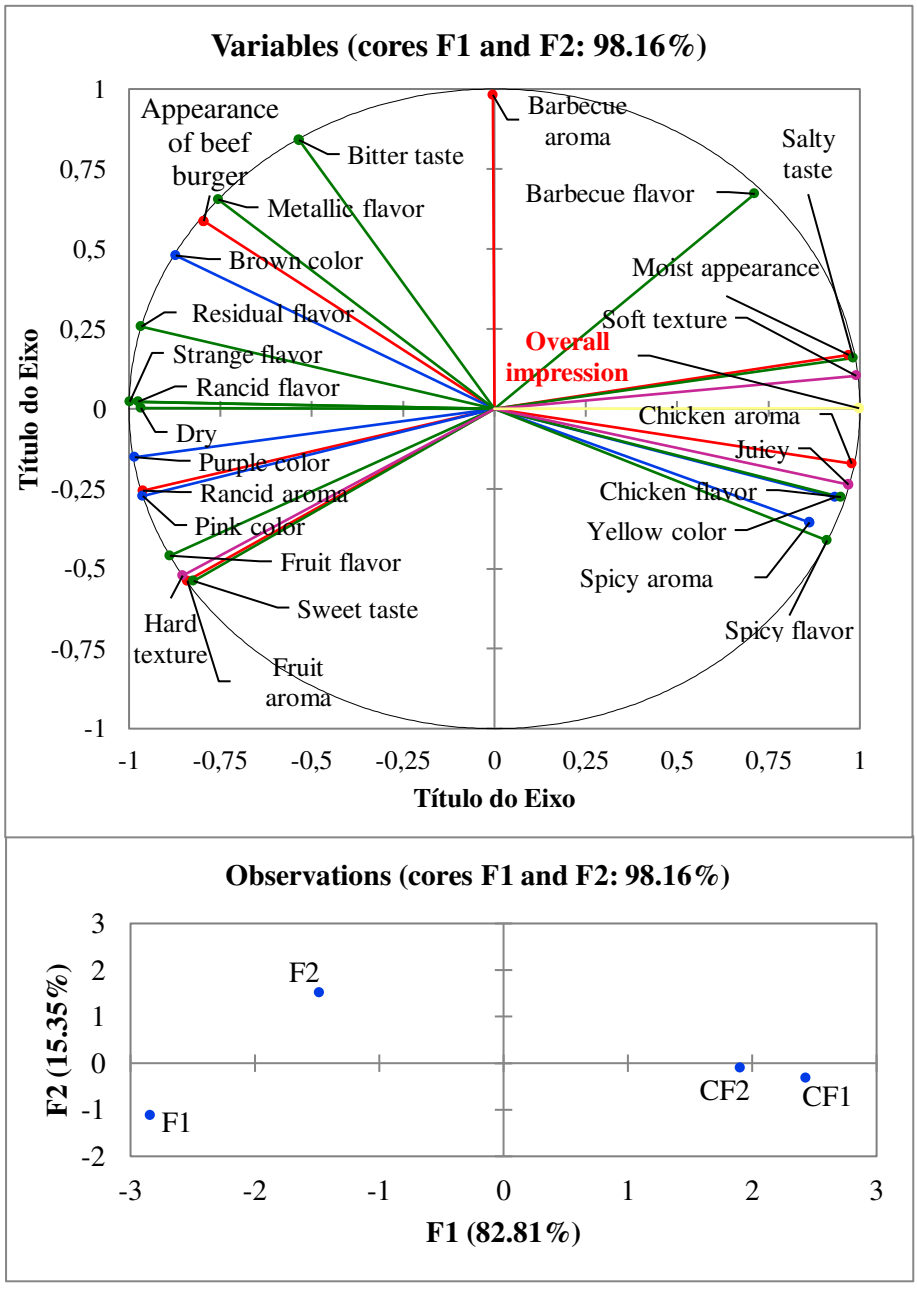


Figure 3. Multiple Factor Analysis of sensory attributes evaluated by CATA for chicken burgers, samples with 0 days of storage.

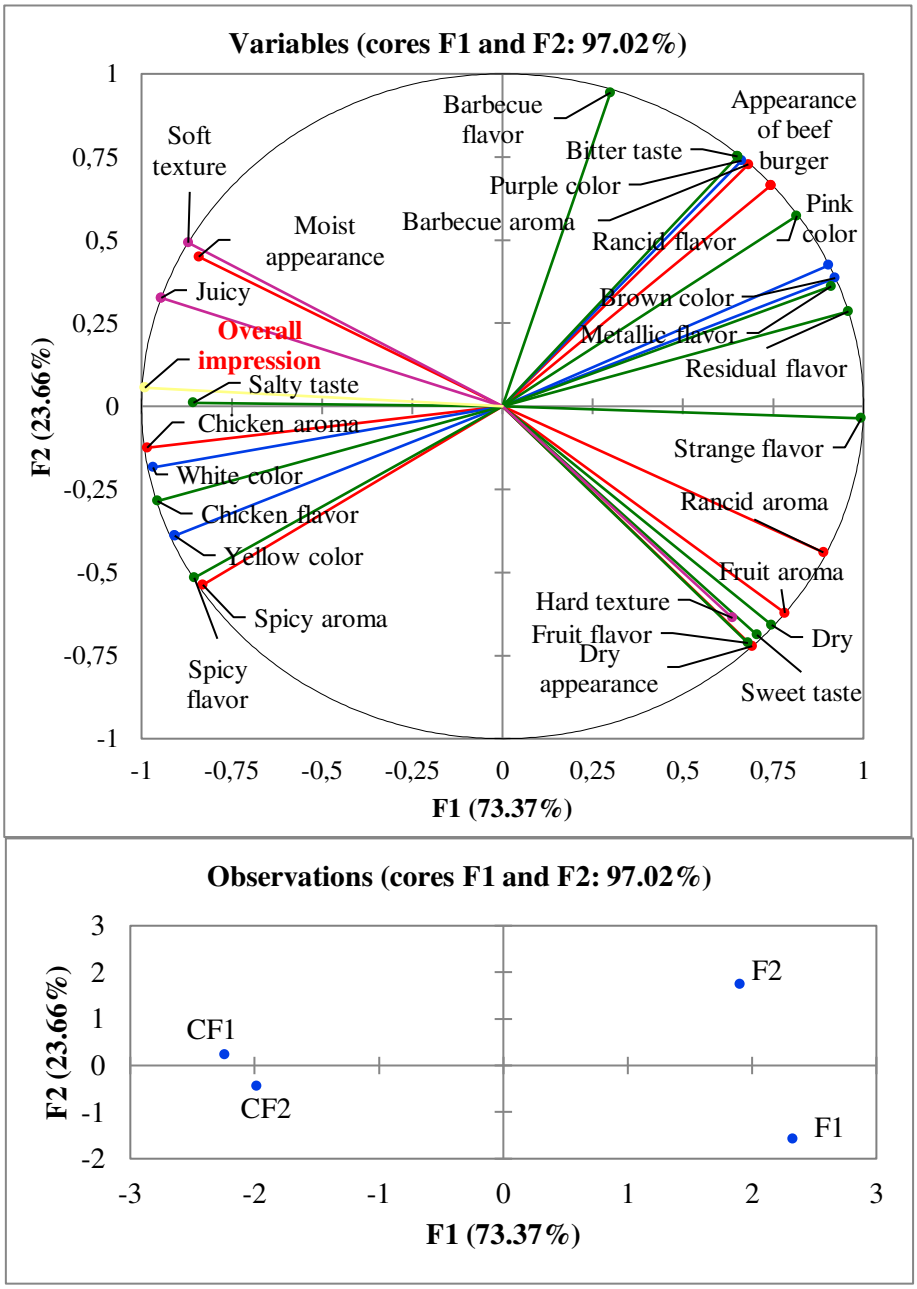


Figure 4. Multiple Factor Analysis of sensory attributes evaluated by CATA to chicken burgers with samples with 60 days of storage.

F1 and F2 treatments were positioned on the opposite side of the overall impression in two MFA analyses, in 0-day and 60-day times of storage of the chicken burger. It is noteworthy that the most important attributes in F1 treatment (with addition of RB) in 0-day time did not include the purple color; but it was included in samples with 60-day time of storage. This fact may be due to

loss of color compounds, as a result of the interaction between phenolic compounds and other matrix meat compounds, and to oxidation reactions (Jakobek, 2015).

Furthermore, samples right after processing of the F2 treatment (with addition of BB) were evaluated with the attributes of *appearance of beef burger* and *brown color*. However, for samples with 60 days of storage, characteristics of a traditional beef burger were reinforced with new attributes such as: *brown color, barbecue aroma, barbecue flavor, and appearance of beef burger*. This fact may suggest a new possibility of application of blackberry by-products in the reformulation of chicken burgers, but with technological improvement to lessen the attributes bitter flavor, metallic flavor, and residual flavor for the F2 treatment.

Andrade et al. (2018) evaluated sensory and hedonic perceptions of consumers of an innovative meat product, sheep meat copa. 202 people participated in their study, in which they conducted the acceptance testing using the hedonic scale of 9 points, and participants responded the CATA test, composed of 16 sensory terms, which were selected based on a preliminary test with 10 evaluators. Results of the acceptance testing were measured by analysis of variance (ANOVA), and the means were compared by Tukey test at the 5% significance level ($P<0.05$). The authors determined the frequency of the attributes evaluated in CATA and analyzed the significant differences among the samples for each of the sensory terms with the Cochran's Q test. Data on CATA were analyzed by correlation analysis in order to obtain a two-dimensional reproduction of samples. They concluded that information about the product and the reduction of sodium on the label did not change the perception of the participants, but influenced in sensory description, and there is a need to reformulate the meat product before releasing it to the market.

4. Conclusion

Application of raspberry and blackberry pulp processing by-products in chicken burger had an effect of inhibition of lipid oxidation during storage time, and suggest the possibility of

being used as healthier ingredient with antioxidant function instead of synthetic food additives. Regarding sensory analysis, data indicating moderate acceptance of chicken burger with addition of raspberry and blackberry by-products, suggesting that there is still the challenge to improve the sensorial characteristics for a better acceptance by the consumers. In the evaluation of CATA data, it is advised to take advantage of the positive characteristics of the addition of raspberry and blackberry by-products into chicken burgers to conduct new studies for its better use as healthier ingredient.

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DISCUSSÃO GERAL

Os resultados referentes à composição centesimal dos subprodutos do processamento de polpa de framboesa (SF) e amora-preta (SA) foram: conteúdo de proteínas de 9,55 e 12,26 g/100g, respectivamente. O conteúdo de lipídeos do SF foi maior que do SA (20,61 e 14,67 g/100g respectivamente) devido ao fato de SF estar constituído principalmente por semente, aumentando significativamente o conteúdo de lipídeos (Gouw et al., 2017, Radocaj, Vujasinovic, Dimic & Basic, 2014). O conteúdo de fibras totais em SF e SA foi de 54,41 g e 55,85 g respectivamente, e o conteúdo de fibras insolúveis foi de 36,42 g e 24,95 g respectivamente. Os resultados da composição química sugerem que os SF e SA podem ser utilizados como ingrediente alimentar para enriquecer o conteúdo nutricional e o teor de fibras dietéticas benéficas para a saúde (Gil-Sánchez et al., 2018).

Não houve diferença no valor de pH dos SF e SA (3,77 e 3,64 respectivamente). Os valores de atividade de água no SF e SA foram de 0,31 e 0,40 respectivamente. Houve diferença nos valores de acidez titulável entre SF e SA, que foram de 0,57% e 0,65% respectivamente.

Houve diferença nas médias nos valores de L^* (luminosidade), a^* (vermelho/verde), b^* (amarelo/azul), Chroma (saturação) e Hue-angle (ângulo de matiz) nas amostras de SF e SA. Pantelidis et al. (2007) reportaram que diferenças nas características físico-químicas de uma mesma fruta ocorrem em função de diferenças de variedade, condições climáticas no cultivo e condições de armazenamento fruta. Segundo Fischer, Carle & Kammerer (2013), quanto mais alta a temperatura de secagem dos subprodutos de fruta, maior a degradação dos compostos de cor.

Com relação à capacidade de retenção de água (CRA), SA apresentou maior valor que SF (4,26 g contra 3,41 g água/g subproduto em base seca, respectivamente). A diferença nos valores da CRA entre as amostras pode estar relacionada com o conteúdo de fibras dietéticas nas amostras de SF e SA (Biswas, Kumar, Bhosle, Sahoo, & Chatli, 2011).

O conteúdo de antocianinas, fenólicos totais e a capacidade antioxidante aplicada pelo teste de descoloração pelo radical ABTS no SF foi de: 35,87 mg equivalentes de cianidina/100 g de subproduto em base seca, 26,76 mg equivalentes de ácido gálico/g subproduto em base seca, e 699,33 µmol equivalentes de Trolox/g SF em base seca respectivamente. O conteúdo de antocianinas, fenólicos e capacidade antioxidante aplicada pelo teste de descoloração pelo radical ABTS no SA foi de: 342,69 mg equivalentes de cianidina/100g de subproduto em base seca, 33,05 mg equivalentes de ácido gálico/g de subproduto em base seca, e 781,49 µmol equivalentes de Trolox/g SA em base seca respectivamente. A determinação dos compostos fenólicos é relevante

para estimar o potencial antioxidante e as suas possíveis aplicações na reformulação de alimentos (Viljanen, Kylli, Kivikari, & Heinonen, 2004).

Os resultados referentes à composição centesimal dos hambúrgueres de frango foram: o tratamento FC1 (hambúrguer controle com ascorbato de sódio) apresentou maior umidade em comparação com os outros tratamentos. A diferença pode ser em função da adição de ascorbato de sódio na matriz cárnea, que aumenta levemente a acidez, diminuindo o valor de pH, e como consequência, aumenta a capacidade de retenção de água (Feiner, 2006). O conteúdo de lipídeos foi maior nos tratamentos F1 (hambúrguer com adição de SF) e F2 (hambúrguer com adição de SA), quando comparado com os tratamentos controle FC1 (hambúrguer com adição de ascorbato de sódio) e FC2 (hambúrguer sem adição de antioxidante), possivelmente pela presença de lipídios nas sementes que compõe os SF e SA (Radocaj et al., 2014).

Observou-se redução do valor de pH das amostras de hambúrguer de frango com adição de SF e SA, possivelmente em função da moagem dos SF e SA, a qual libera os ácidos graxos provenientes das sementes, elevando a acidez do hambúrguer de frango (Radocaj et al., 2014).

Os tratamentos FC1 e FC2 cozidos apresentaram mudanças significativas nos valores *L**, *a**, *b**, Chroma e Hue-angle. Isso pode significar que aconteceram reações de oxidação lipídica e da mioglobina durante o tempo de armazenamento (Soyer et al., 2010). Em contrapartida, temos que os parâmetros de cor das amostras dos tratamentos F1 e F2 permaneceram estáveis ao longo do tempo de armazenamento, possivelmente devido à presença de compostos antioxidantes provenientes dos SF e SA, evitando mudanças na cor como consequência de oxidação lipídica e proteica (Radocaj et al., 2014).

Os tratamentos F1, F2, F3 e F4 apresentaram os maiores valores no conteúdo de fenólicos totais (4,59; 3,43; 3,11 e 3,24 mg equivalente de ácido gálico/g de hambúrguer de frango em base seca, respectivamente), quando comparados com os tratamentos FC1, FC2, FC3 e FC4 (2,24; 1,78; 1,37 e 1,07 mg equivalente de ácido gálico/g de hambúrguer de frango em base seca, respectivamente). Assim também, os tratamentos F1, F2, F3 e F4 apresentaram valores de atividade antioxidante pelo teste do radical ABTS de 93,9; 80,87; 65,62 e 45,01 µmol equivalentes de Trolox/g hambúrguer de frango em base seca, respectivamente, e para os tratamentos FC1, FC2, FC3 e FC4 os valores de ABTS foram 71,37; 64,83; 47,33 e 48,06 mg equivalentes de Trolox/g de hambúrguer de frango em base seca, respectivamente. Em geral, os tratamentos de hambúrguer de frango com adição de subprodutos do processamento de polpa de framboesa e amora-preta

apresentaram maiores valores de fenólicos totais e capacidade antioxidante quando comparado com os tratamentos controle, exceto no tratamento F4.

Com relação à porcentagem de perda de peso por cocção e à porcentagem de encolhimento do diâmetro das amostras, não houve diferença durante os 60 dias de armazenamento entre os tratamentos. Uma explicação para este fato é que a adição de maltodextrina tem a propriedade de melhorar a capacidade de retenção de água (Feiner, 2006). Os resultados da perda de peso por cozimento foram de 38,06%, 34,85%, 34,84% e 32,98%, e da porcentagem de encolhimento do diâmetro foram de 21,06%, 18,98%, 17,46% e 16,90%, respectivamente para FC2, FC1, F2, F1, demostrando que os tratamentos com adição de SF e SA perderam menos peso e diâmetro durante o cozimento. Os tratamentos controle FC1 e FC2 mantiveram o grau de dureza, elasticidade, coesividade e mastigabilidade, e os tratamentos F1 e F2 apresentaram diminuição significativa dos valores de dureza, coesividade e mastigabilidade.

De forma geral, houve aumento significativo da oxidação lipídica ao longo do tempo de armazenamento nos tratamentos controle (FC1, FC2, FC3 e FC4), o que não foi observado nos tratamentos com adição de subprodutos de framboesa (F1 e F3), cujos valores permaneceram constantes, e nos tratamentos com adição de subprodutos do processamento de amora-preta (F2 e F4), cujos valores apresentaram redução ao longo do tempo de armazenamento. Este fato se deve a que os subprodutos do processamento de framboesa e amora-preta contém compostos fenólicos com potencial antioxidante (Kalt, Forney, Martin, & Prior, 1999; Jacinto-Valderrama et al., no prelo; Souza et al., 2014).

Quanto à análise sensorial, as amostras-controle (FC1 e FC2) obtiveram notas maiores de aceitação em todos os atributos quando comparadas com os outros tratamentos nos dois tempos em que foram realizadas as análises, com termos hedônicos entre “gostei ligeiramente” e “gostei regularmente”. Por outro lado, as amostras com adição de SF e SA apresentaram avaliações entre “nem gostei, nem desgostei” e “gostei ligeiramente”. A aceitação moderada dos tratamentos F1 e F2 por parte dos avaliadores está possivelmente relacionada com a presença de compostos fenólicos e fibras dietéticas contidos nos SF e SA (Jacinto-Valderrama et al., no prelo), que consequentemente, promoveu mudanças nos atributos Aparência, Aroma, Sabor e Textura, modificando o conceito sensorial de hambúrguer de frango tradicional. Os compostos fenólicos podem conferir cor, sabor amargo e sensação de adstringência quando adicionados ao alimento (Dinnella, Recchia, Tuorila, & Monteleone, 2011; Soto-Vaca, Gutierrez, Losso, Xu & Finley, 2012).

Em geral, o teste Q de Cochran mostrou as características mais importantes percebidas pelos avaliadores, sendo que os atributos melhores avaliados nas amostras-controle FC1 e FC2 foram: aroma e sabor de frango, textura macia e suculência. Já para os tratamentos F1 e F2, os atributos com melhor avaliação foram: sabor residual e gosto amargo. Estas informações confirmam o exposto na análise de frequência do teste CATA, e como explicado anteriormente, os atributos gosto amargo, sabor estranho e sabor residual, seco são atributos sensoriais provenientes dos compostos fenólicos (Jakobek, 2015).

CONCLUSÃO GERAL

Os subprodutos do processamento de polpa de framboesa e amora-preta apresentaram características nutricionais, dietéticas e tecnológicas adequadas para a sua aplicação como aditivo alimentar e, adicionalmente, possuem propriedades antioxidantes. A adição de subproduto de framboesa e amora-preta em hambúrguer de frango aumentou o conteúdo de lipídeos, de compostos fenólicos e a capacidade antioxidante, influenciou na cor e, melhorou as características de cozimento e de perfil de textura. A aplicação de subprodutos do processamento de polpa de framboesa e amora-preta em hambúrguer de frango teve efeito de inibição da oxidação lipídica durante o tempo de armazenamento, sugerindo a possibilidade de serem utilizados como ingredientes mais saudáveis com função antioxidante em substituição de aditivos alimentares. Em relação à análise sensorial, foram obtidos dados que indicam aceitação moderada do hambúrguer de frango com adição de subprodutos de framboesa e amora-preta, o que sugere que ainda existe o desafio de aprimorar as características sensoriais para uma melhor aceitação por parte do consumidor. Na avaliação dos dados do CATA, se aconselha aproveitar as características positivas da adição de subproduto de framboesa e amora-preta em hambúrguer de frango para realizar novos estudos para um melhor aproveitamento como ingrediente alimentar.

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ANEXO 1

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Efeito da aplicação de sub-produto do processamento de polpa de framboesa (*Rubus idaeus*) e amora-preta (*Rubus brasiliensis*) na estabilidade oxidativa e sensorial de hambúrguer sem adição de antioxidante

Nome do responsável: Rickyn Alexander Jacinto Valderrama

Número do CAAE: 76442317.1.0000.5404

Você está sendo convidado a participar como voluntário de uma pesquisa. Este documento, chamado Termo de Consentimento Livre e Esclarecido, visa assegurar seus direitos como participante e é elaborado em duas vias, uma que deverá ficar com você e outra com o pesquisador.

Por favor, leia com atenção e calma, aproveitando para esclarecer suas dúvidas. Se houver perguntas antes ou mesmo depois de assiná-lo, você poderá esclarecê-las com o pesquisador. Se preferir, pode levar esse Termo para casa e consultar seus familiares ou outras pessoas antes de decidir participar. Não haverá nenhum tipo de penalização ou prejuízo se você não aceitar participar ou retirar sua autorização em qualquer momento.

Justificativa e objetivos:

A oxidação lipídica em produto cárneo é um dos fatores de qualidade que pode reduzir a vida de prateleira de produtos cárneos refrigerados, podendo afetar o sabor de alimentos re-aquecidos, desenvolvendo o chamado *"warmed-over-flavor"*, e para inibir ou retardar essa oxidação, são utilizados antioxidantes sintéticos, que também têm sido alvo de muitas críticas por estar relacionado com casos de efeitos toxicológicos e cancerígenos. A indústria processadora de polpa de framboesa e amora preta entre outras, gera subprodutos tais como casca, bagaço e semente, que contêm quantidades significativas de fibras dietéticas e compostos fenólicos, ambos benéficos para saúde. Os compostos fenólicos, possuem propriedades antioxidantes e antimicrobianas. Dessa forma, a proposta deste trabalho é estudar o efeito da aplicação de sub-produto do processamento de polpa de framboesa e amora-preta na estabilidade oxidativa e sensorial de hambúrguer sem adição de antioxidante para obter um produto cárneo mais estável.

Procedimentos:

Participando do estudo você está sendo convidado a provar e avaliar 4 amostras de hambúrguer de frango que foram elaboradas na Planta Piloto de Produto Cárneo (DTA/FEA/UNICAMP) seguindo rigorosos normas higiênico-sanitárias, as amostras são seguras (determinado mediante análise microbiológico). As amostras podem conter os seguintes ingredientes (comuns em hambúrguer): peito de frango, óleo de canola, sub-produto de fruta (framboesa, amora-preta), água, cloreto de sódio, ascorbato de sódio e maltodextrina. Para o Teste de Aceitação, você provará e avaliará as amostras recebidas com relação a cor, aroma, sabor e impressão global, e para o teste CATA, marcará qual ou quais atributos listados na ficha caracterizam a amostra recebida. O tempo estimado para a realização dos testes é de 15 (quinze) minutos. As amostras serão servidas e avaliadas individualmente e, entre as amostras, você receberá um biscoito de sal e água filtrada para lavagem da cavidade bucal. Você deverá preencher uma ficha de avaliação para cada amostra.

Desconfortos e riscos:

Não há riscos previstos na participação da pesquisa, porém você **não** deve participar desse estudo se você for menor de 18 ou maior de 50 anos, se não for aluno ou funcionário da UNICAMP, se tiver problema de alergia ou intolerância a quaisquer dos ingredientes citados acima, se tiver algum tipo de restrição por questões religiosas, culturais ou prescrição médica relacionado com o consumo do hambúrguer e/ou os ingredientes utilizados nesta pesquisa. O voluntário terá toda a liberdade para questionamento de qualquer dúvida e esclarecimento sobre a pesquisa a ser realizada bem como poderá deixar de participar da pesquisa a qualquer momento, sem prejuízos à pesquisa. Também não haverá nenhuma forma de reembolso de dinheiro, já que com a participação na pesquisa o voluntário não terá gastos.

Benefícios:

Não haverá benefícios diretos aos participantes referentes à pesquisa. As suas repostas juntamente com as dos demais participantes da pesquisa auxiliarão na geração de conhecimento e retorno social que poderá permitir a inovação e desenvolvimento sustentável da indústria processadora de polpa de fruta e de produto cárneo.

Acompanhamento e assistência:

Se você tiver algum problema relacionado diretamente à sua participação na pesquisa, você deve comunicar o pesquisador responsável, e será encaminhado para o serviço de emergência disponível no Hospital das Clínicas, Rua Vital Brasil, 251, Campinas - SP.

Sigilo e privacidade:

Você terá a garantia de que sua identidade será mantida em sigilo e nenhuma informação será dada a outras pessoas que não façam parte da equipe de pesquisadores. Na divulgação dos resultados desse estudo, seu nome não será citado.

Ressarcimento e Indenização:

Não haverá ressarcimento visto que os participantes estarão na sua rotina de estudo ou trabalho. Você terá a garantia ao direito á indenização diante de eventuais danos decorrentes da pesquisa.

Contato:

Em caso de dúvidas sobre a pesquisa, você poderá entrar em contato com o pesquisador responsável Rickyn Alexander Jacinto Valderrama, endereço: Laboratório de Frutas, Hortaliças e Produtos Açucarados (DTA/FEA/UNICAMP), Rua Monteiro Lobato, 80, Distrito de Barão Geraldo, Campinas – SP, CEP: 13083-862; E-mail: rickynjacinto@gmail.com. Telefone: (19) 3521-4006.

Em caso de denúncias ou reclamações sobre sua participação e sobre questões éticas do estudo, você poderá entrar em contato com a secretaria do Comitê de Ética e Pesquisa (CEP) da UNICAMP das 8:30hs às 11:30hs e das 13:00hs às 17:00 na Rua: Tessália Vieira de Camargo, 126; CEP 13083-887 Campinas – SP; telefone (19) 3521-8936 ou (19) 3521-7187; e-mail: cep@fcm.unicamp.br.

O Comitê de Ética em Pesquisa (CEP):

O papel do CEP é avaliar e acompanhar os aspectos éticos de todas as pesquisas envolvendo seres humanos. A Comissão Nacional de Ética em Pesquisa (CONEP), tem por objetivo desenvolver a regulamentação sobre proteção dos seres humanos envolvidos nas pesquisas. Desempenha um papel

coordenador da rede de Comitês de Ética em Pesquisa (CEPs) das instituições, além de assumir a função de órgão consultor na área de ética em pesquisas.

Consentimento livre e esclarecido:

Após ter recebido esclarecimentos sobre a natureza da pesquisa, seus objetivos, métodos, benefícios previstos, potenciais riscos e o incômodo que esta possa acarretar, aceito participar e declaro estar recebendo uma via original deste documento assinada pelo pesquisador e por mim, tendo todas as folhas por nós rubricadas:

Nome do (a) participante: _____

Contato telefônico: _____

E-mail (opcional): _____

Data: ____/____/____

_____

Assinatura do participante

Responsabilidade do Pesquisador:

Asseguro ter cumprido as exigências da resolução 466/2012 CNS/MS e complementares na elaboração do protocolo e na obtenção deste Termo de Consentimento Livre e Esclarecido. Asseguro, também, ter explicado e fornecido uma via deste documento ao participante. Informo que o estudo foi aprovado pelo CEP perante o qual o projeto foi apresentado e pela CONEP, quando pertinente. Comprometo-me a utilizar o material e os dados obtidos nesta pesquisa exclusivamente para as finalidades previstas neste documento ou conforme o consentimento dado pelo participante.

_____ Data:____/_____/_____

(Assinatura do pesquisador)

ANEXO 2

Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso
Cadastro nº A9EE28F

A atividade de acesso ao Patrimônio Genético/CTA, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**A9EE28F**
Usuário:	**UNICAMP**
CPF/CNPJ:	**46.068.425/0001-33**
Objeto do Acesso:	**Patrimônio Genético/CTA**
Finalidade do Acesso:	**Pesquisa**

Espécie

Rubus brasiliensis

Rubus brasiliensis

Fonte do CTA

CTA de origem não identificável

Título da Atividade:	**EFEITO DA APLICAÇÃO DE SUBPRODUTOS DO PROCESSAMENTO DE POLPA DE FRAMBOESA (Rubus idaeus) E AMORA-PRETA (Rubus brasiliensis) NAS CARACTERÍSTICAS FÍSICO-QUÍMICAS, CAPACIDADE ANTIOXIDANTE, ESTABILIDADE OXIDATIVA E SENSORIAL DE HAMBÚRGUER DE FRANGO**

Equipe

Priscilla Efraim	**UNICAMP**
Rickyn Alexander Jacinto Valderrama	**UNICAMP**
Kazumi Kawasaki Ramos	**UNICAMP**
Maristela Midori Ozaki	**UNICAMP**
Marise Aparecida Rodrigues Pollonio	**UNICAMP**

Resultados Obtidos

Divulgação de resultados em meios científicos ou de comunicação

Identificação do meio onde foi divulgado:	**Dissertação de mestrado: Rickyn Alexander Ja**

Data do Cadastro:	**23/10/2018 23:32:15**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **23:32** de **23/10/2018**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - SISGEN