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

**Characterization and application of fruit byproducts in the development of
beef burger and corn extruded product**

Miriam Mabel Selani

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

**Piracicaba
2015**

Miriam Mabel Selani
Bachelor of Food Science

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and corn extruded product**

Advisor:
Prof^a. Dr^a. **SOLANGE GUIDOLIN CANNIATTI BRAZACA**

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*This work is dedicated to my parents, Antonio and Catsue,
for the education, dedication and love
and for transmitting me peace and serenity*

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Porque eu sou do tamanho do que vejo

E não do tamanho da minha altura...

Alberto Caeiro

E o que vejo são meus sonhos

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RESUMO

Caracterização e aplicação de subprodutos de frutas no desenvolvimento de hambúrguer bovino e extrusado de milho

Este estudo objetivou caracterizar subprodutos de abacaxi, maracujá e manga e selecionar aquele com maior potencial como ingrediente alimentício para ser aplicado em dois produtos: hambúrguer bovino e extrusado de milho. A fibra foi o maior componente presente nos subprodutos de abacaxi e maracujá (>50%) e, devido a isto, eles mostraram considerável capacidade de retenção de água. Os subprodutos de frutas apresentaram teor de compostos fenólicos e atividade antioxidante considerável, especialmente o de manga. Os resultados das contagens microbianas e dos teores de resíduos de agrotóxicos dos subprodutos apresentaram-se dentro dos limites estabelecidos pela legislação brasileira, estando seguros para aplicação em alimentos. Devido ao alto teor de fibra e às propriedades funcionais apresentadas, os subprodutos foram explorados considerando-se este potencial. Dessa forma, a amostra com melhores resultados foi selecionada e avaliada como substituto parcial de gordura em hambúrguer bovino e como fonte de fibras em extrusado de milho. A primeira etapa da aplicação em hambúrguer bovino avaliou cada subproduto em quatro concentrações (1,0, 1,5, 2,0 e 2,5%), juntamente com o óleo de canola, como substitutos parciais de gordura. Todos os subprodutos melhoraram as propriedades de cozimento dos hambúrgueres, especialmente o de abacaxi. A análise sensorial mostrou que cor, odor e aceitação global não foram afetados pelos tratamentos. Através dos resultados, o subproduto de abacaxi, na concentração de 1,5%, foi selecionado para estudos complementares. Subproduto de abacaxi (1,5%) e óleo de canola (5%) foram avaliados como substitutos parciais de gordura animal em hambúrguer bovino com teor reduzido de gordura. Cinco tratamentos foram realizados: convencional (20% gordura) e quatro formulações com 10% de gordura: controle, com subproduto de abacaxi, com óleo de canola, e com subproduto de abacaxi e óleo de canola. A incorporação do subproduto de abacaxi melhorou as propriedades de cozimento e a textura (força de cisalhamento) e a adição do óleo de canola melhorou o perfil lipídico, sem reduzir a vida útil dos hambúrgueres. A análise descritiva quantitativa indicou que o uso dos dois substitutos de gordura em conjunto pode ser uma alternativa adequada para minimizar alterações sensoriais da redução da gordura. Como fase final deste estudo, o potencial da aplicação de subproduto de abacaxi em extrusado de milho visando o aumento do teor de fibras foi avaliado. Durante a extrusão, subproduto (0, 10,5, 21%), umidade (14, 15, e 16%) e temperatura (140 e 160 °C) foram avaliados. A adição do subproduto de abacaxi diminuiu a expansão e a luminosidade, enquanto que aumentou a intensidade de cor vermelha dos extrusados comparados com o controle (0% subproduto/14% umidade/140 °C). Quando a dureza, intensidade de cor amarela, absorção de água e densidade foram comparadas com o controle, não houve efeito da adição de 10,5% sobre os extrusados, indicando que, neste nível, o subproduto de abacaxi pode ser adicionado, sem promover alterações significativas sobre as propriedades do produto final. Os resultados deste estudo destacam o potencial do subproduto de abacaxi no desenvolvimento de novos produtos, trazendo a oportunidade de convertê-los em ingredientes alimentícios com valor agregado.

Palavras-chave: Subproduto de manga; Subproduto de maracujá; Subproduto de abacaxi; Redução de gordura; Fibra alimentar; Óleo de canola; Substituto de gordura; Ingredientes

ABSTRACT

Characterization and application of fruit byproducts in the development of beef burger and corn extruded product

This study aimed to characterize pineapple, passion fruit and mango byproducts and select the material with the greatest potential as food ingredient to be applied in two food products: beef burger and corn extruded product. Fiber was the major component present in pineapple and passion fruit byproducts (>50%), and, due to this, they showed considerable water holding capacity. The fruit byproducts had considerable phenolic content and antioxidant activity, especially the mango byproduct. The results of the microbial counts and the content of pesticides residues of the byproducts were within the limits established by the Brazilian law and were safe for food application. Due to the high fiber amount and the functional properties presented, the byproducts were explored considering this potential. Thus, the sample with the best results was selected and evaluated as partial fat replacer in beef burger and as source of fiber in a corn extruded product. The first step of the beef burger application evaluated each byproduct in four concentrations (1.0, 1.5, 2.0, and 2.5%), along with canola oil, as partial fat replacers. All byproducts improved the cooking properties of the burgers, especially the pineapple. Sensory analysis showed that color, odor and overall acceptance were not affected by the treatments. Based on the results, pineapple byproduct, at the concentration of 1.5%, was selected for further studies. Pineapple byproduct (1.5%) and canola oil (5%) were evaluated as partial animal fat replacers in low-fat beef burger. Five treatments were performed: conventional (20% fat) and four formulations with 10% of fat: control, with pineapple byproduct, with canola oil, and with pineapple byproduct and canola oil. Pineapple byproduct incorporation improved the cooking properties and the texture (shear force) and canola oil addition improved the lipid profile, without reducing the shelf life of the burgers. The quantitative descriptive analysis indicated that the use of the two fat replacers together can be an alternative choice to minimize the sensory alterations of the fat reduction. As the final step of this study, the potential application of pineapple byproduct in a corn extruded product for fiber enhancement was evaluated. During extrusion, byproduct (0, 10.5, and 21%), moisture (14, 15, and 16%) and temperature (140 and 160 °C) were evaluated. Pineapple byproduct addition decreased expansion and luminosity, while increasing redness of the extrudates compared to the control (0% byproduct/14% moisture/140 °C). When hardness, yellowness, water absorption, and bulk density were compared to the control, there was no effect of 10.5% addition on the extrudates, indicating that, at this level, pineapple byproduct could be added without promoting significant alterations in the properties of the final extruded product. The results of this study highlight the potential of pineapple byproduct in the development of new products, bringing the opportunity to convert them into value-added food ingredients.

Keywords: Mango byproduct; Passion fruit byproduct; Pineapple byproduct; Fat reduction; Dietary fiber; Canola oil; Fat replacer; Food ingredients

1 INTRODUCTION

Large amounts of agro-industrial byproducts are generated all over the world. Brazil, having the agribusiness as the most important sector of its economy, strongly contributes to this generation, mainly due to agriculture and agro-industrial activities. Since most of the byproducts generated by these sectors have high concentration of organic material, if inappropriately disposed, they can cause environmental issues. Thus, for economic and environmental reasons, to reuse or recycle byproducts, adding value to them, is always desirable (AGUEDO et al., 2012).

Pineapple world production reached 24.78 million tons and Brazil was the second largest producer in 2013, with 2.48 million tons (FAO, 2013). Besides the fresh fruit, most of its production is used for the manufacture of several products, such as juices, fruit salads, canned fruits and jams. According to Larrauri, Rupérez and Calixto (1997), residues from pineapple processing represent 25-35% of the fruit, and they still have some bioactive compounds. These authors, studying pineapple peel, identified the phenolic compounds myricetin, salicylic acid, tannic acid, trans-cinnamic acid and p-coumaric acid and also described that the byproduct presents high antioxidant activity. Martínez et al. (2012), studying pineapple byproducts, reported high fiber content (75.8% dry basis) with 75.2% and 0.6% corresponding to insoluble and soluble fiber, respectively. The material also showed high water holding capacity and swelling capacity, indicating that it has potential to be used as ingredient in products requiring hydration and viscosity. Bromelain, an enzyme used in the food industry, was extracted from different pineapple byproducts (peel, core, stem and crown) and all of them showed high caseinolytic activity (KETNAWA; CHAIWUT; RAWDKUEN, 2012).

Passion fruit is a popular tropical fruit and Brazil was the largest world producer in 2012, with 776 thousand tons (FNP, 2015). It is estimated that about 40% of the Brazilian production is intended for processing (ROSSI; ROSSI; SILVA, 2001), being the fresh and concentrated juice the most popular products in the market (KULKARNI; VIJAYANAND, 2010). During passion fruit juice extraction, thousands tons of seeds, pulps and peels are produced. Toledo (2013) characterized passion fruit byproduct and reported a significant amount of fiber (64.05% total dietary fiber, 50.35% of the insoluble fraction and 13.70% of the soluble fraction), minerals, especially potassium, and phenolic compounds. Kulkarni and Vijayanand (2010) optimized the pectin extraction from passion fruit peel and obtained high quality pectin, with a yield of 14.8 g/100 g of dried peel. According to the study of López-Vargas et al. (2013), passion fruit byproducts have different substances that present

antioxidant and antibacterial activities and some technological characteristics, such as water holding and swelling capacities.

With a world production exceeding 43 million tons, and a Brazilian production of 1.16 million tons in 2013 (FAO, 2013), mango is one of the most important and widely consumed tropical fruits. About 20% of the production is processed and juices, purées, nectars and canned slices are some of the most consumed products (LOEILLET, 1994). The amount of byproducts generated by the industry is between 40-60% of the total fruit weight (depending on cultivar and processing), and it is composed of peels and seeds (LARRAURI; CEREZAL, 1993). Some studies have evaluated the presence of valuable components in mango byproducts, mainly in peels. Ajila, Bhat and Prasada-Rao (2007) evaluated raw and ripe mango peels and reported polyphenol contents ranging from 55-100 mg/g peel, 45-78% of dietary fiber, carotenoids (387-1520 µg/g fresh peel), vitamins C (188-392 µg/g dry peel) and E (205-509 µg/g dry peel), and significant amount of enzymes, mainly proteases. The same author, now evaluating mango peel extracts, showed that the material exhibited good antioxidant activity (AJILA et al., 2007).

Animal feed and fertilizers are the usual applications of these byproducts. However, the potentially valuable compounds they contain could be used to convert them into value-added food ingredients (AGUEDO et al., 2012). Since according to previous studies mango, pineapple and passion fruit byproducts have significant amount of dietary fiber, their use with this purpose presents great potential.

It is well-known that dietary fiber plays an important role in human health, promoting several physiological and metabolic positive effects. The insoluble dietary fiber acts as a bulking agent, normalizing intestinal motility, preventing constipation, while soluble fiber is associated to decreasing the intestinal absorption of cholesterol and glucose (SCHENEEMAN, 1987). Due to all of these benefits of dietary fiber intake, a tendency in the development of products enriched with fiber or with specific fiber claims has already been observed for some time. In addition to the physiological benefits, fibers also have good technological properties, such as water holding capacity (WHC), oil holding capacity (OHC) and swelling capacity. These characteristics can be useful in products that require hydration, to avoid syneresis, improve yield, stabilize emulsions, and to modify texture and viscosity (ELLEUCH et al., 2011).

Extrusion cooking has been an extensively used technology in the food industry due to its wide range of food products applications, high productivity, relative low cost and lack of effluents (ALTAN; MCCARTHY; MASKAN, 2008). In the extrusion processing, ingredients

are thermomechanically cooked due to high temperature, pressure and shear stress generated in the screw-barrel (ARHALIASS; BOUVIER; LEGRAND, 2003), which promote chemical changes and molecular transformations in the food components, such as gelatinization of starch, denaturation of protein and inactivation of enzymes, microbes and many anti-nutritional factors (BHATTACHARYA; PRAKASH, 1994). As the product is forced through the die, sudden expansion results from the pressure differential (KELLEY; WALKER, 1999), resulting in low density and light in texture products (BIANCHINI et al., 2012).

However, extrusion-cooked products tend to be nutritionally poor, since they are energy dense, and low in health promoting ingredients. Furthermore, the extrusion process itself promotes the gelatinization and depolymerization of the starch, leading to an increase in the amount of easily digestible carbohydrates and resulting in a product with high glycemic index (BRENNAN et al., 2013). Dietary fiber from noncommercial sources, such as vegetable byproducts, has been introduced into extruded products with the attempt to improve their nutritional quality. Altan, McCarthy and Maskan (2008) evaluated the incorporation of tomato pomace in the development of a barley extruded product and reported that products with 2% and 10% of tomato pomace, extruded at 160 °C and 200 rpm, had higher preference levels for the sensory parameters of color, texture, taste and overall acceptability. The study of Stojceska et al. (2008) showed that the addition of cauliflower byproducts in snacks affected some physical parameters, such as expansion, bulk density, color and cell area, but according to the sensory analysis of acceptance, cauliflower byproduct could be added up to 10% in the product. Another study evaluated the use of mushroom byproduct to develop extruded snacks and, according to the results, expansion was not reduced and the potential glycemic response was lowered (BRENNAN et al., 2012). Considering the characteristics of the extrudates, the previous studies and the high versatility of the products, they can be considered an attractive choice for fiber enrichment.

Other food products that offer a good opportunity for fiber application are the meat products. Burger is one of the most popular processed meat products in the world. It is highly accepted and consumed, mainly due to the current increase in the number of fast foods worldwide and due to its convenience and low price (HOOGENKAMP, 1997). It is classified as a restructured product, term that is suitable to products that are partially or completely comminuted and reconstituted. The raw materials, basically meat and fat, are mixed with salt and seasonings (PASSOS; KUYAE, 2002), and then molded into specific shapes.

Burgers are also known by some negative aspects, such as the quantity (20-30%) (JIMÉNEZ-COLMENERO, 2000) and quality of its fat (mostly saturated fatty acids), as well

as the cholesterol content, which are associated with the occurrence of some chronic and cardiovascular diseases (FERNÁNDEZ-GINÉS et al., 2005). In this type of product, the simple reduction of fat and its substitution by water is not acceptable, since it negatively affects juiciness, flavor, and texture (JIMÉNEZ-COLMENERO, 2000). Thus, in order to improve the quality of low-fat meat products and to avoid the mentioned problems, some ingredients have been studied as adjuncts to be incorporated into meat products, acting as animal fat replacers, such as fibers.

According to Jimenez-Colmenero (2000), in the reformulation of meat products it is of fundamental importance address the lipid fraction both quantitatively - fat reduction - and qualitatively - modification of fatty acid composition. Due to this, vegetable oil is another ingredient that has been evaluated as animal fat replacer in meat products. Vegetable oils are free of cholesterol and they have higher ratios of unsaturated (UFA)/saturated (SFA) fatty acids compared with animal fat (CHOI et al., 2009). The incorporation of vegetable oils in meat products can be an alternative to minimize sensory and technological changes of the animal fat reduction, resulting in positive effects on consumer's health, through calorie, fat and cholesterol reduction and increase in the unsaturated fatty acids.

There are few studies evaluating the combined use of fibers and vegetable oils as fat replacers in meat products, such as olive, corn, soybean, canola or grape seed oil and rice bran fiber in low-fat meat batters (CHOI et al., 2009) and low-fat frankfurters (CHOI et al., 2010), sunflower seed oil and dietary fiber from *makgeolli* lees in low-fat frankfurters (CHOI et al., 2013), canola-olive oils and rice bran in pork frankfurters (ÁLVAREZ et al., 2012), and olive oil and wakame (seaweed rich in fiber) in beef patties (LÓPEZ-LÓPEZ et al., 2010), which has shown nutritional and/or technological improvements in the products.

Considering this panorama, this study was conducted with the aim to characterize pineapple, passion fruit and mango byproducts as food ingredients and, based on their potentials, select one to be applied in the development of two food products: a corn extruded product enriched with fiber and a low-fat beef burger with fiber and canola oil as partial fat replacers.

Thus, the thesis originated six chapters. The first one is an introduction about the study. In the second chapter, pineapple, passion fruit and mango byproducts were characterized through physicochemical, functional and antioxidant properties, as well as their pesticides residues content and microbial counts. In the third chapter, the three fruit byproducts (in different concentrations) and canola oil were evaluated as partial fat replacers in low-fat beef burger regarding physicochemical, sensory and cooking characteristics. This

study was a screening step conducted in order to select the fruit byproduct and the concentration with the greatest potential for application as fat replacer in beef burger. After the selection of the fruit byproduct, low-fat beef burgers with addition of pineapple byproduct and canola oil were evaluated regarding cooking properties, physicochemical, texture and sensory characteristics (fourth chapter), as well as their lipid composition, oxidative stability and cholesterol content (fifth chapter). Finally, the sixth chapter evaluated the potential application of pineapple byproduct in a corn extruded product for fiber enhancement.

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2 PHYSICOCHEMICAL, FUNCTIONAL AND ANTIOXIDANT PROPERTIES OF TROPICAL FRUITS BYPRODUCTS

Abstract

The aim of this study was to determine the physicochemical, functional and antioxidant properties of mango (MAB), pineapple (PAB) and passion fruit (PFB) byproducts in order to evaluate them as ingredients for food application. Proximate composition of the fruit byproducts showed low fat content, and in PAB and PFB total dietary fiber was the major component, representing more than 50% of the freeze-dried samples. Low pH (4.03 – 4.20), water activity (0.20-0.25) along with high acidity (1.36-2.02 g citric acid/100 g dry basis - db) indicated that these freeze-dried byproducts would not be easily susceptible to deterioration as food ingredients. Pineapple and passion fruit byproducts had significant water holding capacity (4.96-4.31 g water/g sample db, respectively), however oil holding capacity was low (1.59-1.85 g oil/g sample db) for all three matrices studied. MAB, PAB and PFB were not good emulsifying agents, but they may be useful for maintaining the integrity of emulsions. Regarding the phenolic content of the byproducts, values ranged from 3.78 to 4.67 mg gallic acid equivalent/g db, with PAB showing the lowest content, followed by PFB and MAB. As observed for the phenolic content, the highest antioxidant activity was found in MAB when measured by both DPPH (29.16 g Trolox/g db) and ABTS (35.81 g Trolox/g db) methods. The results indicated that the fruit byproducts under evaluation could be used as functional ingredient to provide dietary fiber and natural antioxidants to food products. This potential new application brings the opportunity to convert these byproducts into value-added food ingredients.

Keywords: Byproducts; Pineapple; Mango; Passion fruit; Dietary fiber; Natural antioxidants; Phenolic compounds; Functional ingredient

2.1 Introduction

In 2010, the world production of the major tropical fruits was estimated as 62 million tons, representing an increase of 15 million tons compared to previous data of 1998 (FAO, 2010). According to FAO (2011), the increase in trade volumes of tropical fruits is related to the fact that they can be a viable alternative for diversification of traditional crops, which have experienced downward trends in prices. Furthermore, the worldwide increase in value of these fruits has been associated to their exotic sensory attributes and nutritional characteristics (BICAS et al., 2011).

Pineapple, passion fruit, and mango are popular tropical fruits, consumed both fresh and processed, mainly as juices, pulps, and jams. During these processes, large amounts of byproducts are generated, which, if inappropriately treated or disposed, can cause environmental problems. Consequently, from both economic and environmental perspectives, it is important to reuse industrial residues, reducing the cost of their treatment and adding value to these materials.

According to recent studies, fruits and vegetable byproducts have high potential for reutilization, since most of them still contain many valuable substances, such as phenolic compounds and dietary fiber (DF) (UCHOA et al., 2009). Traditionally, the fiber used as functional ingredient is obtained, mostly, from cereals. However, other plant materials could also be used as potential sources of DF, such as fruits and vegetables (MCKEE; LATNER, 2000), which have the advantage of containing considerable amounts of antioxidant compounds.

Dietary fiber is known as an important compound in the human diet, since it provides numerous positive physiological functions. Insoluble dietary fiber (IDF) plays a role in the intestinal tract, acting as a bulking agent, normalizing intestinal motility; while soluble dietary fiber (SDF) influences the metabolism of available carbohydrates and lipids, decreasing their intestinal absorbance (SCHENEEMAN, 1987). Furthermore, fibers have technological properties, such as water and oil holding capacity. These characteristics can be useful in products that require hydration, to avoid syneresis, improve yield, stabilize emulsions, and also to modify texture and viscosity (ELLEUCH et al., 2011).

Antioxidants are substances capable of preventing oxidative damage caused by free radicals (FLORA, 2009). Produced as secondary metabolites in plants, these compounds are used for pharmaceutical, cosmetic, and nutritional purposes. The global trend toward the use of natural components as food ingredients and the current concern about the safety and toxicity of synthetic additives have led to an increase in the research about natural antioxidants, such as those found in agro-industrial byproducts.

Thus, the aim of this study was to determine the physicochemical, functional and antioxidant properties of mango, pineapple and passion fruit byproducts in order to evaluate them as potential ingredients for food application.

2.2 Material and Methods

2.2.1 Material

The byproducts were obtained from a commercial facility located in Jundiaí, SP, Brazil that produces more than one hundred different products, such as frozen fruits and vegetables, juices and pulps. At this facility, fruits were sanitized with 200 ppm of sodium hypochlorite, rinsed with water and then passed through the pulp extractor, where the byproducts of mango (pomace), passion fruit (peel, albedo and seed) and pineapple (peel and pomace) were collected. The samples of these byproducts collected for this research were kept frozen until its transportation to the Laboratory of Food and Nutrition of the “Luiz de

Queiroz” College of Agriculture, University of São Paulo, ESALQ/USP (Piracicaba, SP, Brazil). Upon receiving samples were freeze-dried (EC Modulyo, EC Apparatus Inc., New York, USA) during 96 h, ground using a knife mill (Marconi, Piracicaba, Brazil), passed through a 40-mesh sieve (420 μm) and stored at $-18\text{ }^{\circ}\text{C}$ for further analyses. All the analyses were performed in freeze-dried samples.

2.2.2 Particle size analysis

The particle size distribution of these samples was determined by laser diffraction in a Malvern Mastersizer 3000 (Malvern Instruments, Worcestershire, UK), using the dry dispersion method (Aero S dispersion cell), with a refractive index of 1.53, an air pressure of 4 bar and a sample feed of 50%.

2.2.3 Proximate composition

Moisture, ash, lipid (Soxhlet), and protein (Kjeldahl, $\text{N} \times 6.25$) were determined in triplicate, according to the Association of Official Analytical Chemists - AOAC (2010). Total dietary fiber (TDF), IDF and SDF were determined in triplicate, according to the method described by Asp et al. (1983). Available carbohydrates were calculated by difference. Results were expressed as g/100 g dry basis (db).

2.2.4 Physicochemical analyses

The pH was measured in 10% (w/v) aqueous solutions of the freeze-dried samples (Quimis Q799-02, Diadema, Brazil). Titratable acidity was determined by titration with 0.1N sodium hydroxide and the results were reported as g of citric acid/100 g. Water activity was determined using the Aqualab meter (CX 2T, Decagon devices Inc., Pullman, USA) at $25\text{ }^{\circ}\text{C}$. Color was measured with a colorimeter (Minolta CR-400, Konica Minolta, Osaka, Japan), with an observation angle of 10° and an illuminant C. The parameters were calibrated in a standard white porcelain with $Y=93.7$, $x=0.3160$ and $y=0.3323$ and the following parameters were determined: lightness (L^*), redness (a^*), and yellowness (b^*). All the analyses were performed in triplicate.

2.2.5 Functional properties

Water (WHC) and oil holding capacities (OHC) were determined in triplicate by the method described by Carcea-Bencini (1986), with slight modifications. The byproduct (1 g) was stirred in 10 mL of distilled water (for WHC) or canola oil (for OHC), centrifuged (Nova

Técnica NT 825, Piracicaba, Brazil) at 2200 g for 30 min and the supernatant was carefully eliminated. WHC was reported as grams of water held by gram of sample db and OHC was reported as grams of oil held by gram of sample db.

Emulsifying activity (EA) and emulsion stability (EE) were evaluated according to Sathe, Deshpande and Salunkhe (1983), with modifications. One hundred milliliter of 2% (w/v) sample suspension in water were homogenized in a blender (Waring Products, New Hartford, USA) at 22.000 rpm for 30 s. Canola oil (100 mL) was added to the suspension and blended for another minute. Aliquots of the emulsions were centrifuged at 5000 g for 5 min, and the volume of the emulsion left after centrifugation was measured. The emulsifying activity was calculated as (the volume of the emulsified layer/total sample volume in the centrifuge tube) x 100. To determine the stability, emulsions prepared by the above procedures were heated at 80 °C for 30 min, cooled, and centrifuged at 5000 g for 5 min. The result was calculated as (the volume of the remaining emulsified layer/volume of emulsion obtained after first centrifugation) x 100.

2.2.6 Total phenolic content (TPC) and antioxidant activity (AA)

2.2.6.1 Extraction of total phenolic compounds

Samples (1 g) were extracted with 10 mL of solvent (ethanol:water, 80:20, v/v) for 20 min in an ultrasonic bath (Ultra Cleaner, Unique, Indaiatuba, Brazil), centrifuged (15 min, 5000 g), and the supernatant was collected to perform the analyses of TPC and AA. All samples were extracted in triplicate.

2.2.6.2 Total phenolic content

Total phenolic content was performed using Folin-Ciocalteu spectrophotometric method (SINGLETON; ORTHOFER; LAMUELA-RAVENTÓS, 1999). An aliquot of 0.5 mL of the extracts was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent. After 3 min, 2 mL of sodium carbonate (4% w/v) were added and the tubes were kept in the dark, at room temperature, for 2 h. The absorbance of the final solution was measured at 740 nm (Unico 2800 spectrophotometer, Dayton, USA). Gallic acid was used to build a calibration curve for quantification and the results were expressed as mg gallic acid equivalent (GAE)/g sample db.

2.2.6.3 Antioxidant activity

The DPPH assay was performed according to the method described by Mensor et al. (2001). The reaction mixture consisted of 0.5 mL of standards or extracts, 3.0 mL of ethanol,

and 0.3 mL of DPPH radical (0.5 mM) in ethanol solution. The mixture was incubated in the dark, at room temperature, for 45 min. The absorbance of the final solution was measured at 517 nm and the antioxidant activity reported as $\mu\text{mol Trolox/g}$ of sample db, based on a calibration curve built using Trolox as standard.

The ABTS assay was carried out according to Re et al. (1999), with modifications. For this test, the ABTS radical was formed by reacting 7 mM ABTS with 140 mM potassium persulfate, both in water, followed by incubation at 25 °C in the dark, for 12–16 h. Once formed, the radical was diluted with ethanol to an absorbance of 0.700 ± 0.020 at 734 nm. The sample extract (30 μL) was added to 3.0 mL of ABTS radical and the mixture was kept in the dark for 6 min. The absorbance of the final solution was read at 734 nm, using ethanol as a blank. Trolox was used as reference and the results were calculated as $\mu\text{M Trolox/g}$ sample db.

2.2.7 Determination of pesticides residues

The pesticides residues were quantified in pineapple, passion fruit and mango freeze-dried fruit byproducts. The multiresidue analysis of pesticides was performed using a gas chromatograph coupled with a mass spectrometer (GC-MS) and a liquid chromatograph coupled with a mass spectrometer (LC-MS). The dithiocarbamates and ethephon were determined using a gas chromatograph with a flame photometric detector (GC-FPD). The analyses were performed according to the NBR ISO/IEC 17025 (ABNT, 2001). The investigated pesticides and their maximum residue limits (MRLs) were established according to the “Programa de Análises de Resíduos de Agrotóxicos em Alimentos” (PARA) (BRASIL, 2015a) and with the pesticide monographs (BRASIL, 2015b), both from the “Agência Nacional de Vigilância Sanitária”.

2.2.8 Microbiological analyses

E.coli and thermotolerant coliforms were counted by plating samples on *E.coli*/Coliform Petrifilm plates (3M, St. Paul, USA; recognized as an AOAC official method) following incubation at 45 °C for 24 h. Molds and yeasts counts were determined after plating samples on Dichloran Rose Bengal Chloramphenicol agar (Acumedia, Lansing, USA) and incubating at 25 °C for 5 days (BEUCHAT; COUSIN, 2001). *Salmonella* testing was performed by a pre-enrichment with BPW at 37 °C for 24 h, followed by a selective enrichment with Tetrathionate Broth (Acumedia, Lansing, USA) at 41 °C for 24 h. Enriched

samples were applied to pathogen-specific test strips (RapidCheck, SDIX Newark, USA; validated by the AOAC), according to the manufacturer's directions.

2.2.9 Statistical analysis

A completely randomized design, with 3 replicates per treatment, was used. Analysis of variance and Tukey's test were performed using the SAS software (Version 9, SAS Institute Inc., North Carolina, USA). Correlations between TPC and the two methods of AA were determined using Pearson's correlation coefficient test.

2.3 Results and Discussion

The appearance of the fresh and processed (freeze-dried, ground and sieved) byproducts is shown in Figure 1.

Figure 1 - Appearance of the fruit byproducts. A: fresh pineapple byproduct; B: fresh passion fruit byproduct; C: fresh mango byproduct; D: freeze-dried pineapple byproduct; E: freeze-dried passion fruit byproduct; F: freeze-dried mango byproduct

2.3.1 Particle size analysis

According to the particle size distribution of the freeze-dried byproducts, it is possible to observe that the grinding and sieving processes (40 mesh = 420 μm) were efficient, since approximately 85% of the pineapple and mango byproducts and 92.96% of the passion fruit byproduct showed particle size smaller than 454 μm (Table 1). This previous analysis is important since some functional properties, such as water and oil holding capacity, are influenced by the particle size of the samples. More finely ground samples typically retain less water and oil than samples with larger diameters.

Table 1 - Particle size distribution of freeze-dried pineapple, passion fruit and mango byproducts

Size (μm)	% Undersize particles ^a		
	PAB	PFB	MAB
211	42.14 $\pm$ 0.55	57.67 $\pm$ 0.72	54.05 $\pm$ 2.11
454	84.85 $\pm$ 0.88	92.69 $\pm$ 1.37	85.05 $\pm$ 3.28
666	96.40 $\pm$ 0.75	98.95 $\pm$ 1.05	91.10 $\pm$ 3.56
859	98.86 $\pm$ 0.62	99.54 $\pm$ 0.79	92.61 $\pm$ 3.51
1110	99.37 $\pm$ 0.55	99.73 $\pm$ 0.47	93.78 $\pm$ 3.14
3500	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00

Mean $\pm$ Standard deviation.

^aPercentage of particles with diameter under the size (μm) described in the first column.

PAB: pineapple byproduct; PFB: passion fruit byproduct; MAB: mango byproduct.

2.3.2 Proximate composition

Among the three byproducts evaluated in this study, PFB showed significantly higher contents of SDF, protein, lipid, and ash (Table 2). According to a previous report, passion fruit seeds have 24.5 g lipids per 100 g (CHAU; HUANG, 2004), which could explain the highest content of lipid in PFB. In the study of Kulkarni and Vijayanand (2010), the level of pectin in passion fruit peel was measured at 14.8 g of pectin/100 g, showing that, similarly to apple and citrus byproducts, passion fruit peel also has high contents of pectin. This helps explaining the highest SDF content observed in this byproduct when compared to the other ones evaluated in the present study.

Table 2 – Proximate composition (g/100 g db) of freeze-dried pineapple, passion fruit and mango byproducts

Component	PAB	PFB	MAB
Moisture	6.60 $\pm$ 0.23 ^a	5.85 $\pm$ 0.28 ^b	6.12 $\pm$ 0.24 ^{ab}
Protein	4.00 $\pm$ 0.02 ^c	10.08 $\pm$ 0.18 ^a	7.42 $\pm$ 0.09 ^b
Lipid	1.32 $\pm$ 0.10 ^b	5.64 $\pm$ 0.015 ^a	0.95 $\pm$ 0.10 ^c
Ash	2.45 $\pm$ 0.01 ^b	5.24 $\pm$ 0.10 ^a	2.43 $\pm$ 0.02 ^b
SDF	1.90 $\pm$ 0.14 ^c	8.90 $\pm$ 0.51 ^a	6.54 $\pm$ 0.15 ^b
IDF	67.75 $\pm$ 0.014 ^a	48.05 $\pm$ 1.02 ^b	25.20 $\pm$ 0.22 ^c
TDF	69.64 $\pm$ 0.28 ^a	56.93 $\pm$ 0.51 ^b	31.57 $\pm$ 0.46 ^c
Carbohydrate*	15.92 ^b	16.26 ^b	51.22 ^a

*Carbohydrate by difference.

Mean $\pm$ Standard deviation.

Values followed by different letters in the same row are significantly different ($p < 0.05$) according to Tukey's test.

PAB: pineapple byproduct; PFB: passion fruit byproduct; MAB: mango byproduct; SDF: soluble dietary fiber; IDF: insoluble dietary fiber; TDF: total dietary fiber; db: dry basis.

The ash content of MAB and PFB is similar to those reported by Larrauri et al. (1996) (2.85 g/100 g) for mango peel and by Martínez et al. (2012) (5 g/100 g) for passion fruit

byproduct. However, in PAB, the present study found 2.45 g/100 g, which is lower than the value reported by a previous study in pineapple byproduct (4.5 g/100 g) (MARTÍNEZ et al., 2012). Differences in fruit variety may influence the ash content, but the main factor determining mineral concentration in plants is the type of soil used for cultivation (LOMBARDI-BOCCIA et al., 2004).

According to the proximate composition, the major component in PAB and PFB was TDF (Table 2). These results are in agreement with those reported by Larrauri, Rupérez and Calixto (1997), who found 70.61 g/100 g of TDF in pineapple shell (70.10 g/100 g IDF and 0.51 g/100 g SDF) and by López-Vargas et al. (2013), which reported TDF value of 53.51 g/100 g (48.25 g/100 g IDF and 5.26 g/100 g SDF) in passion fruit byproduct. Mango byproduct presented TDF value of 31.57 g/100 g, which is higher than the value reported by García-Magaña et al. (2013), for Tommy Atkins pulp pomace (22.50 g/100 g).

When MAB was compared to the other two matrices evaluated, it presented lower TDF and the highest amount of carbohydrates (Table 2), probably because this byproduct was mainly pulp pomace, without the presence of seeds and peels, which are normally richer in fiber.

Thus, according to the proximate composition of pineapple, passion fruit and mango byproducts, they could potentially be used as functional ingredients for fiber enrichment.

2.3.3 Physicochemical analyses

The pH of PFB was 4.03 (Table 3), which is within the values observed by López-Vargas et al. (2013), for passion fruit albedo (4.36) and passion fruit seed and peel (3.75). The pineapple byproduct had a pH slightly higher than the results found by Selani et al. (2014) (chapter 6) in pineapple pomace (3.86) and this difference was probably due to the use of a different pineapple variety, since Selani et al. (2014) used the variety Gold and in the present study the byproducts were obtained from Smooth Cayenne pineapples. Among the three byproducts, mango presented the highest pH, which was similar to the pH of Tommy Atkins mango (4.56-4.83) (SABATO et al., 2009), the variety used by the processing plant from where the byproduct was sourced.

Table 3 - Physicochemical characteristics of freeze-dried pineapple, passion fruit and mango byproducts

Sample	pH	Water activity	Titratable acidity	Color		
				L*	a*	b*
PAB	4.08±0.01 ^b	0.21±0.00 ^b	1.90±0.07 ^a	73.45±1.20 ^b	0.59±0.10 ^b	20.65±0.49 ^c
PFB	4.03±0.02 ^c	0.20±0.01 ^b	2.02±0.18 ^a	74.48±1.02 ^b	1.31±0.16 ^a	28.62±0.74 ^b
MAB	4.20±0.02 ^a	0.25±0.00 ^a	1.36±0.07 ^b	81.09±0.26 ^a	0.61±0.20 ^b	42.18±0.85 ^a

*Titratable acidity (g citric acid/100 g)

Mean ± Standard deviation.

Values followed by different letters in the same column are significantly different ($p < 0.05$) according to Tukey's test.

PAB: pineapple byproduct; PFB: passion fruit byproduct; MAB: mango byproduct.

The results obtained for water activity (Table 3), as expected, were considered low due to the water removal during the freeze-drying process. The values of the present study are in agreement with those previously reported for passion fruit albedo (0.16) and passion fruit seed and peel (0.21), which was dried at 60 °C for 24 h (LÓPEZ-VARGAS et al., 2013), and for pineapple core dietary fiber (0.24), dried at 70 °C, overnight (PRAKONGPAN; NITITHAMYONG; LUANGPITUKSA, 2002). Based on their pH and water activity, the three byproducts evaluated seemed to be at low risk for deterioration by microorganisms, enzymes or nonenzymatic reactions.

The acidity levels found in MAB was significantly lower than the values obtained for PFB and PAB (Table 3). Other studies reported acidity values of 1.63 g/100 g in passion fruit peel (MARTÍNEZ et al., 2012), 1.99 - 2.98% in mango peel (SOGI et al., 2013), and 2.01 g citric acid/100g in pineapple pomace (SELANI et al., 2014). Variations in acidity levels may be due to factors such as variety, agricultural practices, growing seasons, but mainly because of variations in fruit ripening, since according to Sudheer and Indira (2007), the acid content of fruits usually decreases during ripening due to utilization of organic acids during respiration or their conversion to sugars.

When color was evaluated, MAB showed the highest L* value (81.09) (Table 3), indicating that its color was lighter than the other byproducts. This difference in lightness may have been caused by the presence of peel in PAB and peel and seed in PFB; while MAB had just pulp pomace in its composition. For comparison purposes, Aziz et al. (2012) reported a L* value of 60.99 for ripe mango pulp flour, which is lower than the result found in this study; while López-Vargas et al. (2013) reported a L* value of 79.91 for passion fruit albedo, which is similar to the ones reported here (74.48). Color is influenced by many factors including fruit variety and ripeness, but in the case of the MAB, the drying method could have been the main factor. In the work reported by Aziz et al. (2012), a hot-air drier (60 °C/24h)

was used, which may induce enzymatic and non-enzymatic reactions (Maillard), leading to darkening of the product. When the matrices evaluated by this study are compared with other fruit byproducts, they have higher L^* values than pomegranate bagasse (62.2–62.8) (VIUDAMARTOS et al., 2012), and lemon juice byproducts (46.83–68.97) (LARIO et al., 2004). This attribute may be considered positive, since ingredients with dark color could limit potential food applications.

For the red-green coordinate (a^* value), the results of PAB (0.59) and MAB (0.61) were similar but different from PFB (1.31) (Table 3). Similar a^* values to the ones reported here for PFB (1.53–2.96) were found by López-Vargas et al. (2013). Among the matrices evaluated, the highest b^* value was measured in MAB (pulp pomace). This indicates that this byproduct has a more pronounced yellowish color compared to the others, which was expected due to the mango pulp natural color.

2.3.4 Functional properties

There were significant differences in the WHC among the three fruit byproducts, where pineapple had the highest value, followed by passion fruit and mango (Table 4). A similar result was also observed for TDF content, which explains the WHC trend, since one of the functional properties of fiber is its capacity to absorb water. Because of their ability to hold water, PAB and PFB could have potential to be used as functional ingredients in products requiring hydration, to avoid syneresis, improve yield, and modify texture and viscosity (ELLEUCH et al., 2011).

Previous studies have reported WHC of 6.40 g water/g sample in mango peel (70 % TDF) (MARTÍNEZ et al., 2012), 13.00 g water/g sample in passion fruit albedo (71.79 % TDF) (LÓPEZ-VARGAS et al., 2013) and 6.00–9.92 g water/g sample in pineapple core (95.60 % TDF) (PRAKONGPAN; NITITHAMYONG; LUANGPITUKSA, 2002), which are all higher than the values observed here. As previously discussed, fiber has the ability to absorb water and the differences in byproducts composition among studies could lead to distinct values of WHC. Indeed, the levels of TDF should explain the lower WHC observed in this study, since byproducts reported here had all less than 70% TDF. Furthermore, the chemical structure of the polysaccharides, porosity, particle size, ionic form, extraction condition, pH, IDF/SDF ratio, temperature, ionic strength, type of ions in solution, plant source and stresses upon fibers can all influence the hydration properties of dietary fibers (ELLEUCH et al., 2011).

Table 4 - Functional properties of freeze-dried pineapple, passion fruit and mango byproducts

Sample	WHC (g water/g sample db)	OHC (g oil/g sample db)	EA (mL/100 mL)	ES (mL/100 mL)
PAB	4.96±0.03 ^a	1.85±0.01 ^a	2.54±0.13 ^c	84.59±6.45 ^b
PFB	4.51±0.09 ^b	1.57±0.03 ^b	9.53±0.35 ^b	97.29±0.83 ^a
MAB	2.87±0.03 ^c	1.59±0.03 ^b	10.31±0.23 ^a	85.89±1.95 ^b

Mean ± Standard deviation.

Values followed by different letters in the same column are significantly different ($p < 0.05$) according to Tukey's test.

PAB: pineapple byproduct; PFB: passion fruit byproduct; MAB: mango byproduct; WHC: water holding capacity; OHC: oil holding capacity; EA: emulsifying activity; EE: emulsion stability; db: dry basis.

As observed for WHC, the pineapple byproduct also showed higher OHC when compared to PFB and MAB (Table 4). According to Thebaudin et al. (1997), OHC correlates with the IDF content, which explains the results reported here (Table 4), since PAB had the highest content of IDF. Previously reported data on the OHC of mango byproduct (1.6 g oil/g sample db) (MARTÍNEZ et al., 2012) is in agreement with that measured in this study (1.59 g oil/g sample db) (Table 4). Similarly, the values reported here for OHC of PFB and PAB is within those reported ranges of 0.9 to 2.03 g oil/g db for passion fruit byproducts (MARTÍNEZ et al., 2012; LÓPEZ-VARGAS et al., 2013) and of 0.7 to 2.44 g oil/g db for pineapple byproducts (PRAKONGPAN; NITITHAMYONG; LUANGPITUKSA, 2002; MARTÍNEZ et al., 2012). The small differences observed for OHC among studies could be due to factors influencing this property, such as surface properties, overall charge density and thickness (LÓPEZ et al., 1996). The results found in the present study for this functional property can be considered low when compared to pomegranate bagasse (5.9 g oil/g sample db) (VIUDA-MARTOS et al., 2012), lemon byproducts (6.60-6.71 g oil/g sample db) (LARIO et al., 2004), and asparagus byproducts (5.28-8.53 g oil/g sample db) (FUENTES-ALVENTOSA et al., 2009).

Regarding the emulsifying activity, all the matrices were different from each other (Table 4) and showed values considered low when compared to other fruit byproducts, such as pomegranate (30.7-37.3 mL/100 mL) (VIUDA-MARTOS et al., 2012) and chia byproducts (56 mL/100 mL) (CAPITANI et al., 2012). These low values can be explained by the low protein content in the samples (4.00-10.08 g/100 g), which is known to be a good emulsifying agent. However, when emulsion stability was evaluated, the byproducts showed high values, ranging from 84.59 to 97.29 mL/100 mL. These values are similar to the results reported for pomegranate bagasse (90.7-93.8 mL/100 mL) (VIUDA-MARTOS et al., 2012) and are higher than the values found for chia byproduct (60 mL/100 mL) (CAPITANI et al., 2012). Although pineapple, passion fruit and mango byproducts do not have properties as emulsifying agents,

the ES results are indicative that they may be useful for maintaining the integrity of emulsions.

2.3.5 Determination of total phenolic content and antioxidant activity

The results for TPC showed that MAB had higher content compared to PAB and PFB (Table 5), with the last two showing similar values. Martínez et al. (2012), while evaluating ethanolic extracts of mango, guava, pineapple and passion fruit byproducts, found a similar trend for TPC, with pineapple showing the least and mango the highest amounts. However, in general, the values reported by Martínez et al. (2012) were lower than the ones reported here. The difference in values between studies could be due to the different methods of drying, extraction and protocol of analysis. Furthermore, phenolic content can be influenced by maturity, cultivars, horticultural practices, geographic origin, postharvest storage conditions and processing parameters (GOBBO-NETO; LOPES, 2007). Comparing the phenolic content with the amount found in other vegetable byproducts, the fruit byproducts studied here have higher values than guava pomace (3.41 mg GAE/g db) (PACKER et al., 2015), pear byproduct (2.25 mg GAE/g db), and dry date byproduct (2.78 mg GAE/g db) (AGUEDO et al., 2012), but lower values compared to fig byproduct (4.78-5.76 mg GAE/g sample db) (VIUDA-MARTOS et al., 2015), wine industry byproducts (7.84-9.41 mg GAE/g sample db) (SELANI et al., 2011), and pomegranate bagasse (10.05 g GAE/g sample db) (VIUDA-MARTOS et al., 2011).

The antioxidant activity as determined by the DPPH assay showed that pineapple and passion fruit byproducts were not different (Table 5) and both had much lower activity than MAB. Similar results were reported by Martínez et al. (2012). According to the results shown in Table 5, the antioxidant activity by DPPH increased with the phenolic content, which was confirmed by the high and positive correlation between these two measurements ($r=0.99$).

Table 5 - Total phenolic content and antioxidant activity of freeze-dried pineapple, passion fruit and mango byproducts

Sample	Total phenolic content (mg GAE/g db)	DPPH (μ mol Trolox/g db)	ABTS (μ mol Trolox/g db)
PAB	3.78 $\pm$ 0.05 ^b	5.76 $\pm$ 0.12 ^b	13.46 $\pm$ 0.62 ^b
PFB	3.86 $\pm$ 0.03 ^b	6.28 $\pm$ 0.05 ^b	13.65 $\pm$ 0.40 ^b
MAB	4.67 $\pm$ 0.13 ^a	29.16 $\pm$ 0.76 ^a	35.81 $\pm$ 1.01 ^a

Mean $\pm$ Standard deviation.

Values followed by different letters in the same column are significantly different ($p < 0.05$) according to Tukey's test.

PAB: pineapple byproduct; PFB: passion fruit byproduct; MAB: mango byproduct.

For the ABTS method, the same tendency among the samples was observed, with MAB showing an activity approximately 2.6 times higher than PAB and PFB. The study of Martínez et al. (2012), evaluating tropical fruit byproducts also observed the same pattern for mango, passion fruit, and pineapple. Comparing the AA of the byproducts evaluated here with the pulp of these fruits studied by Prado (2009), MAB had a higher antioxidant activity than its pulp (18.7 $\mu\text{mol Trolox/g db}$); however the antioxidant activity of pineapple (18.7 $\mu\text{mol Trolox/g db}$) and passion fruit (25 $\mu\text{mol Trolox/g db}$) pulps exceeded the results of their byproducts as reported here. Once again, there was a strong correlation ($r = 0.99$) between TPC and AA as measured by ABTS, showing that the higher the phenolic content, the higher the antioxidant activity.

2.3.6 Determination of pesticides residues

The multiresidue analysis of pesticides evaluated 239 compounds. None of these compounds was detected in pineapple byproduct. In mango byproduct, 0.313 mg carbendazim/kg of byproduct was quantified. According to PARA (BRASIL, 2015a) and the pesticides monographs (BRASIL, 2015b), the MRL of carbendazim is 2 mg/kg of mango, indicating that MAB showed values below those recommended by the legislation. Regarding passion fruit byproduct, the compounds acephate (0.323 mg/kg), carbendazim (2.164 mg/kg), dithiocarbamate (<0.01 mg/kg), methamidophos (0.598 mg/kg) and profenofos (0.215 mg/kg) were found.

According to the Normative Instruction number 1 (June 16, 2014), regulated by the “Ministério da Agricultura, Pecuária e Abastecimento” (MAPA), by the “Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais”, and by the “Agência Nacional de Vigilância Sanitária” (BRASIL, 2014), passion fruit is a minor crop, i.e., it is one of the crops with insufficient phytosanitary support. Thus, the guidelines for registering pesticides and MRLs are extrapolated to a representative crop, which, in the case of passion fruit, are citrus (*Citrus sp.*) and melon (*Cucumis melo*).

For orange, PARA indicates as MRLs the amounts of 0.5 mg acephate/kg, 5 mg carbendazim/kg, and 2 mg dithiocarbamates/kg. Methamidophos is not allowed for the crop, however, according to PARA, when acephate and methamidophos are identified in the same sample, it is not considered unsatisfactory, since methamidophos may occur as a result of the degradation of acephate. Regarding profenofos, this is authorized for watermelon, which belongs to the same family of melon, and its MRA is 0.5 mg/kg. According to these results,

PFB showed pesticides values below the MRLs. It is still important to consider that the concentrations found here would be even lower after the incorporation in a food product.

Thus, according to the pesticides identified and quantified in freeze-dried samples, they can be considered safe for consumption regarding the presence of pesticides residues.

2.3.7 Microbiological analyses

All fruit byproducts evaluated had counts <1 log CFU/g (<10 CFU/g) for thermotolerant coliforms and *E. coli*. Coliforms and enterococci are part of the normal flora of plant products, and populations of 10^2 or 10^3 CFU per g of processed products are not uncommon (BRACKETT; SPLITTSTOESSER, 2011). Yeasts counts of pineapple, passion fruit, and mango byproducts were 1.30, <1 , and 1.90 log CFU/g, respectively, and molds counts were 2.23, 1.48, and 1.30 log CFU/g, respectively. Since both microorganisms are more tolerant to low pH and acid condition, fungi are often predominant in fruit products and additional measures may be necessary to prevent them from spoiling. Regarding *Salmonella*, its absence in 25 g of pineapple, passion fruit and mango byproducts indicates the safety regarding this specific batch of products evaluated. Besides, the counts were within the limits established by the RDC 12, of the “Agência Nacional de Vigilância Sanitária” (BRASIL, 2001) for freeze-dried fruits (thermotolerant coliforms: 10^2 CFU/g and absence of *Samonella* in 25 g).

2.4 Conclusions

The three fruit byproducts evaluated in this study showed low fat content along with high amounts of fiber ($>50\%$) in pineapple and passion fruit byproducts. These are important characteristics for ingredients in the development of healthy foods. The low pH, water activity and high acidity measured in the different byproducts indicate low risk of deterioration by microbial or enzymatic activity. Due to their TDF, pineapple and passion fruit byproducts had considerable WHC, indicating their usefulness as functional ingredients in products that require hydration, to improve yield, and to modify texture and viscosity. The fruit byproducts presented phenolic contents ranging from 3.78 (PAB) to 4.67 mg GAE/g sample (MAB) and mango byproduct stood out regarding the antioxidant activity.

The results described in this study indicate that the fruit byproducts evaluated have potential to be used as functional ingredients, providing dietary fiber content (for both nutritional and technological purposes) and/or natural antioxidants to food products. This

potential new application brings the opportunity to convert these byproducts into value-added food ingredients.

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3 PHYSICOCHEMICAL, SENSORY AND COOKING PROPERTIES OF LOW-FAT BEEF BURGERS WITH ADDITION OF FRUIT BYPRODUCTS AND CANOLA OIL

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 SELANI, M.M.; MARGIOTTA, G.B.; PIEDADE, S.M.S.; CONTRERAS-CASTILLO, C.J.; CANNIATTI-BRAZACA, S.G. Physicochemical, sensory and cooking properties of low-fat beef burgers with addition of fruit byproducts and canola oil. **International Proceedings of Chemical, Biological and Environmental Engineering**, v. 18, p. 58-65, 2015.

Abstract

This study evaluated the addition of canola oil and pineapple, passion fruit and mango byproducts on physicochemical, sensory and cooking properties of burgers. Fourteen formulations were performed: conventional (CN) (20% fat) and formulations with 50% of fat reduction (10% fat): control (CT), without canola oil and fruit byproduct; and 12 formulations with canola oil (5%) and pineapple (PA) or passion fruit (PF) or mango (MA) byproducts in four concentrations (1, 1.5, 2, 2.5%). The burgers were analyzed for color, pH, water activity (Aw), cooking loss (CL), moisture retention (MR), fat retention (FR), reduction in diameter (RD), increase in thickness (IT), and sensory characteristics. The byproducts addition decreased CL, RD, IT, and increased MR, indicating improvement in yield and better visual characteristics. Lightness was not affected by the addition of byproducts and canola oil. PA and MA showed lower a^* and higher b^* values, respectively. The higher the amount of byproducts added, the lower the pH. Sensory characteristics were not affected by either the byproduct or the canola oil in the attributes of color, odor and overall acceptance of the burgers. In general, CT had the lowest scores for the attributes. Pineapple byproduct at 1.5% showed the best results as fat substitute in beef burgers.

Keywords: Mango; Passion fruit; Pineapple, Canola oil; Low-fat

3.1 Introduction

Fat has considerable importance in meat products, since it affects technological properties and sensory aspects, mainly tenderness and juiciness. However, diets high in fats provide large amounts of saturated fatty acids (SFA) and cholesterol, which are associated with the occurrence of obesity, hypertension, cardiovascular disease and coronary heart disease (OZUVURAL; VURAL, 2008).

Due to this, the increase of the consumer's concern with health has led to changes in eating habits, resulting in an increased demand for low-fat products. However, in meat products, the simple reduction of fat is not acceptable, mainly regarding juiciness, flavor, and texture (JIMÉNEZ-COLMENERO, 2000).

Thus, in order to develop low-fat meat products of better quality and to avoid the mentioned problems, some ingredients have been studied as adjuncts to be incorporated into

meat products, acting as animal fat replacers. Vegetable oils and fibers are some of these ingredients used to improve sensory and physical characteristics of low-fat meat products.

Vegetable oils are free of cholesterol and they have higher proportion of unsaturated/saturated fatty acids compared to animal fat (CHOI et al., 2009). The incorporation of vegetable oils in meat products can be an alternative to minimize the sensory and technological changes of the animal fat reduction, resulting in positive effects on consumer's health through calorie reduction, decrease in fat and cholesterol and increase in the content of unsaturated fatty acids.

Agro-industrial activities generate millions tons of residues all over the world. Since many of them still contain considerable amount of fiber, they have potential for food application. Byproducts from pineapple, mango and passion fruit processing, due to their high fiber contents, have potential to be studied as a food ingredient to improve the technological properties of the product.

The objective of this study was to evaluate the addition of canola oil and different concentrations of pineapple, passion fruit and mango byproducts rich in fiber on physicochemical and sensory characteristics of low-fat beef burgers.

3.2 Material and Methods

3.2.1 Byproduct preparation

The byproducts of mango (pomace), pineapple (peel and pomace) and passion fruit (peel, albedo and seed) were collected in a fruit pulp industry, freeze-dried, ground, passed through a 40-mesh sieve and stored under -18 °C.

3.2.2 Burger manufacture

Fourteen formulations were performed: conventional (CN), with the usual fat amount of beef burger (20%) and formulations with 50% of fat reduction (10%): control (CT), without canola oil and fruit byproduct; and twelve formulations with canola oil (5%) and pineapple (PA) or passion fruit (PF) or mango (MA) byproduct in four concentrations (1, 1.5, 2, 2.5%) (Table 1).

Table 1 – Formulations of beef burgers with addition of canola oil and fruit byproducts (g/100g)

Treatments	Ingredients						
	Beef meat	Back fat	Cold water	Canola oil	Fruit byproduct	Salt	Mix for burger*
CN	70	20	7.5	-	-	1.5	1
CT	70	10	17.5	-	-	1.5	1
PA 1.0	70	10	11.5	5	1.0	1.5	1
PA 1.5	70	10	11.0	5	1.5	1.5	1
PA 2.0	70	10	10.5	5	2.0	1.5	1
PA 2.5	70	10	10.0	5	2.5	1.5	1
PF 1.0	70	10	11.5	5	1.0	1.5	1
PF 1.5	70	10	11.0	5	1.5	1.5	1
PF 2.0	70	10	10.5	5	2.0	1.5	1
PF 2.5	70	10	10.0	5	2.5	1.5	1
MA 1.0	70	10	11.5	5	1.0	1.5	1
MA 1.5	70	10	11.0	5	1.5	1.5	1
MA 2.0	70	10	10.5	5	2.0	1.5	1
MA 2.5	70	10	10.0	5	2.5	1.5	1

*Commercial mix for burger: salt, maltodextrin, sodium polyphosphate, sodium erythorbate, natural spices and monosodium glutamate.

CN: conventional, with 20% fat; CT: control, with 10% fat;

PA 1.0, PA 1.5, PA 2.0, PA 2.5: formulations with 1.0, 1.5, 2.0 and 2.5% of pineapple byproduct, respectively;

PF 1.0, PF 1.5, PF 2.0, PF 2.5: formulations with 1.0, 1.5, 2.0 and 2.5% of passion fruit byproduct, respectively;

MA 1.0, MA 1.5, MA 2.0, MA 2.5: formulations with 1.0, 1.5, 2.0 and 2.5% of mango byproduct, respectively.

For the burger manufacture, beef meat (with fat removed) and pork back fat were ground (0.8 cm plate). Fat content was determined in both samples in order to adjust the fat amount of the formulations with pork back fat. Beef meat, pork back fat, pineapple byproduct, canola oil (10 parts of oil, 8 parts of water at 50 °C and 1 part of soy protein isolate), salt, the mix of condiments and additives (salt, maltodextrin, sodium polyphosphate, sodium erythorbate, natural spices and monosodium glutamate), and the cold water were manually mixed for 5 min. The doughs (100 g) were shaped (1 cm thick and 10 cm diameter) using a burger shaper. The burgers were stored under -18 °C. The cooking procedure was performed before the analyses, in a hot plate, until the internal temperature of 75 °C.

3.2.3 Cooking properties

Cooking loss was calculated according to the American Meat Science Association - AMSA (AMSA, 1978). Moisture retention, fat retention, reduction in diameter and increase in thickness were performed according to the described by Sánchez-Zapata et al. (2010). All these determinations were performed in three burgers of each treatment.

3.2.4 Physicochemical analysis

Color was determined with a Minolta colorimeter, using the CIELAB system. The pH was performed using a potentiometer with automatic temperature compensation and a glass penetration electrode. Water activity was performed at 25 °C, using Aqualab equipment, according to the manufacturer's directions. All these determinations were performed in three burgers of each treatment.

3.2.5 Sensory characteristics

Sensory analysis was performed using a hedonic scale of 9 points, ranging from 1 (dislike extremely) to 9 (like extremely). Fifty six panelists evaluated the attributes of color, odor, texture, and overall acceptance, in two sessions. The burgers were cooked according to the procedure previously described. The samples were cut into cubes and for the color attribute, a whole burger was presented. The panelists evaluated five samples, which were coded with random numbers of three digits. Since there were 14 treatments, the sensory analysis was performed using a balanced incomplete block design, with 56 panelists evaluating 5 samples, with a total of 20 evaluations of each treatment, in each session. The protocol for sensory analysis was approved by the Ethics Committee of Human Research of the "Luiz de Queiroz" College of Agriculture, University of São Paulo, ESALQ/USP (Piracicaba, SP, Brazil – protocol n° 92).

3.2.6 Statistical analysis

The study was a randomized block design, with two blocks (two independent processes). For the sensory analysis, a balanced incomplete block design was performed due to the number of treatments. Significant differences among samples were determined by analysis of variance (ANOVA) and Tukey's test ($P < 0.05$), using the software SAS.

3.3 Results and Discussion

3.3.1 Cooking properties

The addition of fruit byproducts affected the cooking loss of the burgers (Table 2). As the amount of byproducts added increased, the cooking loss decreased. This tendency was observed for the three samples tested (PA, PF, MA), indicating that the use of fruit byproducts rich in fiber has positive effects on the burger yield. However, significant effect of the cooking loss compared with the conventional burger (CN) was observed just for the treatment PA 2.5%.

This highest yield of burgers with PA, followed by burgers with passion fruit and mango, could be related to the fiber content and water holding capacity (WHC) of the byproducts, which presented the following descending order: pineapple (69.64 g fiber/100 g sample and WHC of 4.96 g water/g sample) > passion fruit (56.93 g fiber/100 g sample and WHC of 4.51 g water/g sample) > mango (31.57 g fiber/100 g sample and WHC of 2.87 g water/g sample) (chapter 2). Similar results were found by López-Vargas et al. (2014), who verified that, compared with control treatment, samples with addition of passion fruit albedo showed an increase in cooking yield and it occurred in a concentration-dependent manner, with no statistical differences among samples.

According to Kastner and Felicio (1980), the meat grinding during burger processing results in a tender product due to the breakdown of the myofibrils and connective tissue, which, however, promotes weight loss during the cooking process. This factor results in the release of fluids, such as water, water-soluble nutrients, pigments and compounds responsible for flavor and odor (MEIRA, 2013). Thus, the addition of fruit byproducts in beef burger, especially pineapple byproduct, showed positive results, increasing yield and reducing the release of important compounds of the product. Treatments with the lowest byproduct amount showed CL values similar to the control, indicating that concentrations equal or below 1.0% do not improve yield.

Table 2 - Cooking properties (%) of burgers with addition of fruit byproducts and canola oil

Treatment	Cooking loss	Moisture retention	Fat retention	Reduction in diameter	Increase in thickness
CN	38.64±1.55 ^{abc}	57.22±2.63 ^{abc}	53.58±3.04 ^b	24.40±0.07 ^a	23.70±4.18 ^{abcd}
CT	42.21±0.84 ^{ab}	46.22±1.19 ^{de}	83.50±0.40 ^a	24.65±4.13 ^a	13.04±4.08 ^d
PA 1.0	41.21±5.82 ^{ab}	46.86±7.84 ^{de}	77.07±8.99 ^{ab}	26.08±2.79 ^a	27.14±2.11 ^{ab}
PA 1.5	34.20±1.34 ^{bc}	54.80±0.82 ^{bcd}	78.97±1.65 ^{ab}	20.63±2.68 ^{ab}	19.20±3.11 ^{bcd}
PA 2.0	30.56±2.54 ^{cd}	59.16±3.50 ^{ab}	82.83±2.96 ^a	19.28±1.62 ^{ab}	19.26±2.81 ^{bcd}
PA 2.5	24.91±4.91 ^d	65.20±5.01 ^a	92.59±8.95 ^a	16.98±0.63 ^b	14.20±0.97 ^{cd}
PF 1.0	43.88±6.50 ^a	43.30±5.81 ^e	75.93±11.57 ^{ab}	24.64±0.89 ^a	20.37±4.05 ^{abcd}
PF 1.5	40.27±2.01 ^{ab}	47.69±2.78 ^{de}	75.53±2.13 ^{ab}	22.74±0.88 ^{ab}	19.61±4.86 ^{abcd}
PF 2.0	36.66±0.36 ^{abc}	51.31±2.83 ^{bcd}	79.54±10.22 ^{ab}	21.40±2.38 ^{ab}	18.09±1.67 ^{bcd}
PF 2.5	35.80±0.25 ^{abc}	52.63±1.34 ^{bcd}	77.24±11.40 ^{ab}	21.72±1.84 ^{ab}	26.80±0.89 ^{ab}
MA 1.0	41.30±2.34 ^{ab}	47.27±3.60 ^{de}	75.71±4.05 ^{ab}	23.83±0.54 ^{ab}	19.79±0.39 ^{abcd}
MA 1.5	38.70±0.22 ^{abc}	50.57±0.59 ^{bcd}	70.91±1.23 ^{ab}	23.97±1.63 ^{ab}	27.91±0.56 ^{ab}
MA 2.0	39.44±1.25 ^{ab}	48.31±1.73 ^{cde}	77.73±1.64 ^{ab}	24.09±2.99 ^{ab}	24.38±1.56 ^{abc}
MA 2.5	37.58±1.65 ^{abc}	51.12±3.71 ^{bcd}	73.48±4.19 ^{ab}	23.79±0.42 ^{ab}	30.38±1.83 ^a

Mean ± Standard deviation.

Different letters in the same column differ significantly ($P<0.05$) by the Tukey's test.

CN: conventional, with 20% fat; CT: control, with 10% fat;

PA 1.0, PA 1.5, PA 2.0, PA 2.5: formulations with 1.0, 1.5, 2.0 and 2.5% of pineapple byproduct, respectively;

PF 1.0, PF 1.5, PF 2.0, PF 2.5: formulations with 1.0, 1.5, 2.0 and 2.5% of passion fruit byproduct, respectively;

MA 1.0, MA 1.5, MA 2.0, MA 2.5: formulations with 1.0, 1.5, 2.0 and 2.5% of mango byproduct, respectively.

Comparing the moisture retention of the treatments with the same byproduct concentration, in general, burgers of the treatment PA showed higher moisture retention, which, in the formulations PA 2.0% and PA 2.5% resulted in significantly higher values than the observed in the conventional treatment (Table 2). For the treatments PF and MA, with exception of PF 2.5%, there was no significant difference ($p>0.05$) among them.

The results observed for moisture retention can also be explained by the property of the fiber to hold water. Since pineapple byproduct has the highest amount of fiber, it consequently showed higher water holding capacity, which, when used as a food ingredient in beef burger, resulted in products with higher percentages of moisture retention. This result is important since high retention of both water and fat, positively influences some characteristics, such as texture and juiciness of meat products.

Aleson-Carbonell et al. (2005), studying lemon albedo and López-Vargas et al. (2014), using passion fruit albedo also observed increase in moisture retention of burgers with addition of ingredients rich in fiber.

Regarding fat retention, conventional treatment presented the lowest value compared to the others (Table 2). According to Besbes et al. (2010), fat retention is related to the capacity of the meat matrix to bind fat. Thus, this lower retention of CN may be occurred because it has twice the fat content, but the same amount of beef meat compared to the other treatments. In other words, the amount of protein derived from the meat may not have been sufficient to retain the added fat (only 53.58 % was retained), whereas, in the other samples, the retention ranged from 70.91 to 92.59%.

Among treatments with the same fat content (control and formulations PA, PF and MA), only those with the highest concentrations of pineapple byproduct had higher, but not significant values ($p>0.05$), compared to the control. This result can be explained by the low oil holding capacity of the freeze-dried byproducts, which varied from 1.57 to 1.85 g oil/100 g sample (chapter 2).

Burgers with addition of pineapple byproduct showed the lowest reduction in diameter and only the treatment PA 2.5% had significant difference compared with CN and CT (Table 2). The reduction in diameter is a result of meat protein denaturation with loss of water and fat during cooking (LÓPEZ-VARGAS et al., 2014). Thus, since pineapple byproduct showed the highest water and oil holding capacity and resulted in burgers with higher moisture and fat retention, these facts consequently led to products with higher diameters.

The results obtained are in concordance with the study of Turhan, Sagir, and Ustun (2005) with low-fat beef burgers incorporated with hazelnut pellicle and with the study of Tekin, Saricoban, Yilmaz (2010), in burgers with addition of wheat bran.

In the formulations PA and PF, the increase of the byproduct concentration and a consequent increase of the fiber content in the burgers, probably resulted in compensation of the dimensional parameters, leading to burgers with higher diameters and lower thickness. According to the study of López-Vargas et al. (2014) with passion fruit albedo, the same behavior was observed in pork burgers, which, according to the author, could be attributed to binding properties and stabilization of the passion fruit albedo, limiting the distortion of the products during cooking.

3.3.2 Physicochemical properties

Color evaluation showed that neither the fat reduction nor the addition of fruit byproducts and canola oil significantly affected the L* value of both raw and cooked burgers (Table 3). These results differ from those obtained by López-Vargas et al. (2014) in pork burger with addition of passion fruit albedo and those obtained by Turhan, Sagir and Ustun (2005) in beef burger with hazelnut pellicle.

The fact that the luminosity of the samples was not altered by the treatments is considered positive, since in meat and meat products, color is an important quality attribute and one of the main factors determining the acceptability by the consumer, which can be influenced by the concentration and chemical state of myoglobin, the physical characteristics of the meat and the presence of non-meat ingredients (HUNT; KROPF, 1987).

In relation to the a* value, it was not strongly influenced by the addition of fruit byproducts, since only the treatments with higher levels of pineapple byproduct showed significant reduction in redness compared to the conventional treatment. This result may be due to the influence of the pineapple byproduct color on the natural color of the meat, since according to Table 3, its addition promote the lowest L *, a * and b * values. Decreases in a* values were also reported by Turhan, Sagir and Ustun (2005) in beef burger with hazelnut pellicle and by Choi et al. (2012) in low-fat pork burger with addition of seaweed (*L. japonica*).

Table 3 - Color of raw and cooked burgers with addition of fruit byproducts and canola oil

Treatments	Raw Burger			Cooked Burger		
	L*	a*	b*	L*	a*	b*
CN	51.00±4.61 ^a	21.95±0.96 ^a	14.55±1.73 ^a	47.57±0.88 ^a	8.22±0.01 ^a	11.73±0.33 ^{de}
CT	47.57±0.80 ^a	20.25±2.85 ^{ab}	13.81±2.20 ^a	50.08±1.34 ^a	7.90±0.18 ^{ab}	11.33±0.32 ^e
PA 1.0	47.59±0.83 ^a	18.60±0.36 ^{abc}	14.37±0.48 ^a	50.35±1.87 ^a	7.39±0.18 ^{abc}	11.69±0.73 ^{de}
PA 1.5	47.90±0.45 ^a	18.67±0.89 ^{abc}	15.61±0.51 ^a	50.06±1.97 ^a	6.78±0.07 ^{bcd}	11.84±0.65 ^{cde}
PA 2.0	47.22±0.51 ^a	17.36±0.20 ^{bc}	15.28±0.21 ^a	49.42±2.88 ^a	6.13±0.74 ^d	12.29±0.50 ^{bcd}
PA 2.5	50.05±3.14 ^a	15.80±0.41 ^c	16.06±0.35 ^a	51.59±0.27 ^a	6.30±0.46 ^{cd}	13.41±0.10 ^{abc}
PF 1.0	50.60±2.31 ^a	18.51±0.13 ^{abc}	14.26±0.65 ^a	51.14±0.23 ^a	7.69±0.64 ^{ab}	12.94±0.16 ^{bcd}
PF 1.5	50.70±1.35 ^a	18.82±0.01 ^{abc}	15.07±0.01 ^a	51.24±1.46 ^a	7.63±0.61 ^{ab}	13.20±0.07 ^{abcd}
PF 2.0	51.27±1.67 ^a	18.66±0.86 ^{abc}	14.89±0.14 ^a	51.06±1.31 ^a	7.27±0.13 ^{abcd}	13.04±0.35 ^{abcd}
PF 2.5	51.26±0.57 ^a	18.22±1.28 ^{bc}	15.32±0.10 ^a	51.15±0.50 ^a	7.31±0.10 ^{abcd}	13.50±0.04 ^{ab}
MA 1.0	50.77±1.23 ^a	19.93±1.58 ^{ab}	15.05±0.62 ^a	48.63±1.75 ^a	7.41±0.25 ^{abc}	13.04±0.58 ^{abcd}
MA 1.5	51.19±3.15 ^a	19.03±1.39 ^{abc}	15.17±0.02 ^a	48.73±3.02 ^a	7.49±0.13 ^{abc}	13.14±0.65 ^{abcd}
MA 2.0	51.39±0.46 ^a	19.13±0.76 ^{abc}	15.79±0.38 ^a	49.15±2.71 ^a	7.76±0.20 ^{ab}	13.75±0.34 ^{ab}
MA 2.5	51.81±0.97 ^a	19.03±0.71 ^{abc}	16.45±0.17 ^a	48.56±2.68 ^a	7.77±0.28 ^{ab}	14.60±0.28 ^a

Mean ± Standard deviation.

Different letters in the same column differ significantly ($P < 0.05$) by the Tukey's test.

CN: conventional, with 20% fat; CT: control, with 10% fat;

PA 1.0, PA 1.5, PA 2.0, PA 2.5: formulations with 1.0, 1.5, 2.0 and 2.5% of pineapple byproduct, respectively;

PF 1.0, PF 1.5, PF 2.0, PF 2.5: formulations with 1.0, 1.5, 2.0 and 2.5% of passion fruit byproduct, respectively;

MA 1.0, MA 1.5, MA 2.0, MA 2.5: formulations with 1.0, 1.5, 2.0 and 2.5% of mango byproduct, respectively.

Cooked burgers with 2.5% of fruit byproducts showed significant increase in the intensity of yellow compared to CN (20% fat) and CT (10% fat without addition of fruit fiber and canola oil). The addition of mango byproduct promoted the greatest change in b^* value, reflecting the influence of the byproducts color, which have b^* values of 42.18, 20.65 and 28.62 for mango, pineapple and passion fruit, respectively (chapter 2). The raw products did not present significant effect ($p>0.05$) for yellowness.

There was significant effect of the addition of fruit byproducts on the pH of the beef burgers, both raw and cooked (Table 4).

Table 4 - pH values and water activity of raw and cooked burgers with addition of fruit byproducts and canola oil

Treatments	pH		Aw	
	Raw Burger	Cooked Burger	Raw Burger	Cooked Burger
CN	6.12±0.25 ^a	6.49±0.14 ^a	0.97±0.00 ^a	0.96±0.00 ^a
CT	6.04±0.08 ^{ab}	6.45±0.23 ^{ab}	0.98±0.00 ^a	0.96±0.00 ^a
PA 1.0	5.93±0.11 ^{abc}	6.42±0.25 ^{ab}	0.97±0.00 ^a	0.95±0.01 ^a
PA 1.5	5.89±0.12 ^{abcd}	6.43±0.23 ^{ab}	0.97±0.00 ^a	0.96±0.00 ^a
PA 2.0	5.88±0.09 ^{bcd}	6.36±0.23 ^{ab}	0.97±0.00 ^a	0.96±0.00 ^a
PA 2.5	5.86±0.13 ^{bcd}	6.32±0.22 ^{bc}	0.97±0.00 ^a	0.96±0.00 ^a
PF 1.0	5.94±0.18 ^{abc}	6.40±0.25 ^{ab}	0.97±0.00 ^a	0.95±0.00 ^a
PF 1.5	5.81±0.11 ^{cd}	6.30±0.24 ^{bc}	0.97±0.00 ^a	0.96±0.00 ^a
PF 2.0	5.75±0.12 ^{cd}	6.17±0.14 ^{dc}	0.97±0.00 ^a	0.95±0.01 ^a
PF 2.5	5.68±0.12 ^d	6.13±0.19 ^d	0.97±0.00 ^a	0.95±0.00 ^a
MA 1.0	5.97±0.06 ^{abc}	6.45±0.23 ^{ab}	0.97±0.00 ^a	0.96±0.00 ^a
MA 1.5	5.90±0.05 ^{abcd}	6.41±0.20 ^{ab}	0.97±0.00 ^a	0.96±0.00 ^a
MA 2.0	5.77±0.17 ^{dc}	6.35±0.20 ^{ab}	0.97±0.00 ^a	0.95±0.00 ^a
MA 2.5	5.84±0.02 ^{bcd}	6.31±0.14 ^{bc}	0.97±0.00 ^a	0.96±0.00 ^a

Mean ± Standard deviation.

Different letters in the same column differ significantly ($P<0.05$) by the Tukey's test.

CN: conventional, with 20% fat; CT: control, with 10% fat;

PA 1.0, PA 1.5, PA 2.0, PA 2.5: formulations with 1.0, 1.5, 2.0 and 2.5% of pineapple byproduct, respectively;

PF 1.0, PF 1.5, PF 2.0, PF 2.5: formulations with 1.0, 1.5, 2.0 and 2.5% of passion fruit byproduct, respectively;

MA 1.0, MA 1.5, MA 2.0, MA 2.5: formulations with 1.0, 1.5, 2.0 and 2.5% of mango byproduct, respectively.

In general, it was observed that the higher the amount of fruit byproduct added, the lower the pH of the samples, and this decrease was significant compared to CN only in the formulations with the highest levels of addition. Among the treatments, the lowest pH values were observed in those with passion fruit byproduct, followed by PA and PF. These results were certainly influenced by the characteristics of each byproduct, since according to a previous experiment (chapter 2) passion fruit presented the lowest pH and the highest acidity among the byproducts studied.

Comparing the pH results of the raw and cooked products, it could be observed that the cooking process increased the pH of the samples. According to Choi et al. (2012), the pH increases when meat products are heated because imidazolium, a base in the histidine amino acid, is exposed during cooking due to protein denaturation.

The results of this study are in agreement with the study of Aleson-Carbonell et al. (2005) with beef burger and lemon albedo. However, the study of Gök et al. (2001) did not find effect of poppy seeds addition on the pH of beef burgers.

Regarding the A_w values, despite the fact that the fruit byproducts studied here has the capacity to retain water, this property did not significantly affect ($p>0.05$) the water activity of both raw and cooked burgers, which is an important result since high values of A_w could favor microbial growth. There was reduction in the A_w of the cooked burgers compared to raw products, which was expected, since during cooking process, part of the water is eliminated.

3.3.3 Sensory characteristics

The results of the acceptance test were positive, since it was not observed significant effect ($p>0.05$) of the addition of different fruit byproducts and canola oil in the attributes of color, odor and overall acceptance of beef burgers (Table 5).

Table 5 - Sensory attributes of burgers with addition of fruit byproducts and canola oil

Treatment	Color	Odor	Texture	Overall Acceptance
CN	6.20±0.21 ^a	6.10±0.35 ^a	6.10±0.49 ^{ab}	5.95±0.14 ^a
CT	6.17±0.32 ^a	5.75±0.35 ^a	4.31±0.20 ^b	5.68±0.10 ^a
PA 1.0	6.20±0.00 ^a	6.43±0.32 ^a	6.62±0.09 ^a	6.13±0.11 ^a
PA 1.5	6.10±0.21 ^a	6.48±0.39 ^a	6.63±0.67 ^a	6.00±0.49 ^a
PA 2.0	6.69±0.08 ^a	6.59±0.06 ^a	6.95±0.22 ^a	6.72±0.32 ^a
PA 2.5	6.88±0.95 ^a	6.28±0.17 ^a	6.73±0.04 ^a	6.78±0.88 ^a
PF 1.0	6.80±0.36 ^a	6.59±0.19 ^a	5.28±0.31 ^{ab}	6.39±0.62 ^a
PF 1.5	6.88±0.46 ^a	6.02±0.11 ^a	4.98±1.23 ^{ab}	6.50±0.42 ^a
PF 2.0	6.33±0.88 ^a	5.97±0.12 ^a	5.84±0.65 ^{ab}	5.90±0.85 ^a
PF 2.5	7.15±0.49 ^a	6.25±0.85 ^a	6.50±0.00 ^{ab}	6.68±0.81 ^a
MA 1.0	6.62±0.46 ^a	6.67±0.31 ^a	5.93±0.89 ^{ab}	6.24±0.41 ^a
MA 1.5	6.38±0.18 ^a	6.63±0.18 ^a	6.11±0.15 ^{ab}	6.38±0.46 ^a
MA 2.0	6.30±0.64 ^a	6.83±0.04 ^a	6.43±0.04 ^{ab}	6.15±0.85 ^a
MA 2.5	6.70±0.07 ^a	6.62±0.02 ^a	6.08±0.25 ^{ab}	6.45±0.21 ^a

Mean ± Standard deviation.

Different letters in the same column differ significantly ($P<0.05$) by the Tukey's test.

CN: conventional, with 20% fat; CT: control, with 10% fat;

PA 1.0, PA 1.5, PA 2.0, PA 2.5: formulations with 1.0, 1.5, 2.0 and 2.5% of pineapple byproduct, respectively;

PF 1.0, PF 1.5, PF 2.0, PF 2.5: formulations with 1.0, 1.5, 2.0 and 2.5% of passion fruit byproduct, respectively;

MA 1.0, MA 1.5, MA 2.0, MA 2.5: formulations with 1.0, 1.5, 2.0 and 2.5% of mango byproduct, respectively.

For the texture attribute, panelists indicated the lowest score for the control treatment (4.31), which is between the “indifferent” (5) and “slightly dislike” (4) terms. Fat strongly influences the texture and juiciness of meat products and since CT treatment had the reduction of 50% of fat without the addition of fiber and vegetable oil, this slight rejection could be due to hardness and low juiciness of the product.

There was no significant difference ($p>0.05$) among treatments with byproducts addition for the texture attribute. However, samples with PA had slightly higher scores, which may be due to higher moisture retention of the product, positively affecting tenderness and juiciness. Despite of the highest scores, treatments with 2.0 and 2.5% of pineapple byproducts presented problems of cracking and breakage after cooking, probably due to the high water retention of the fibers.

The treatments MR had the lowest texture scores, which may have been a consequence of the high amount of soluble fiber in the passion fruit byproduct (8.90 g/100 g sample) (chapter 2). Soluble fibers bind to water and form gels, which may have promoted some elasticity and resistance to chewing, negatively affecting the texture of the burger.

Except for color, control treatment had the lowest scores in the other attributes, which is in agreement with the fact that fat reduction of meat products, by simply decreasing its quantity, is not acceptable from the sensory point of view.

3.4 Conclusions

The addition of byproducts positively affected the cooking properties of beef burgers. Among the byproducts studied, treatments with pineapple byproduct showed the best results, leading to a decrease in the parameters of cooking loss, reduction in diameter, and increase in thickness and an increase in the moisture retention, resulting in improvement in yield and better visual characteristics.

Lightness and water activity were not affected by the presence of fruit byproducts and canola oil. Formulations with higher amounts of pineapple and mango byproducts showed lower a^* values and higher b^* values, respectively. However, in the sensory analysis of color, panelists did not detect color alterations.

Sensory characteristics showed positive results, since there was no effect of either byproducts or canola oil addition in the attributes of color, odor and overall acceptance of the burgers. In general, control treatment had the lowest scores for the attributes evaluated, confirming that the simple reduction of fat is not sensory acceptable. The texture was affected by the addition of fruit byproducts, with the treatment PA presenting slightly higher scores.

According to the cooking properties, the physicochemical, and the sensory characteristics, pineapple byproduct, at the concentration of 1.5%, showed the best results as fat substitute in beef burgers.

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4 EFFECT OF PINEAPPLE BYPRODUCT AND CANOLA OIL AS FAT REPLACERS ON PHYSICOCHEMICAL AND SENSORY QUALITY OF LOW-FAT BEEF BURGER

Abstract

Pineapple byproduct and canola oil were evaluated as fat replacers on physicochemical, texture and sensory characteristics of low-fat beef burgers. Four low-fat formulations (10%) were evaluated: control (CT), with pineapple byproduct (PA), with canola oil (CO), and with pineapple byproduct and canola oil (PC), which were compared to the conventional burger (CN, 20% fat). Higher water and fat retention and consequently lower cooking loss and diameter reduction were found in burgers with byproduct addition. In raw burgers, pineapple byproduct incorporation reduced L^* , a^* , and C^* values, but the cooking process masked these alterations, leading to products similar to the conventional burgers. Low-fat treatments were harder, chewier, and more cohesive than the full-fat treatment. However, according to the Warner Bratzler shear force analysis, PA and PC were as tender as CN. In the quantitative descriptive analysis, there was no difference between CN and PC in any of the sensory attributes evaluated. Pineapple byproducts along with canola oil are promising ingredients as fat replacers in beef burgers.

Keywords: Fat-replacer; Texture profile analysis; Quantitative descriptive analysis; Physicochemical analysis

4.1 Introduction

Diets high in fats provide large amounts of saturated fat and cholesterol, which are associated with several chronic diseases. Due to this, changes in population's diet have been observed, with an increase in preference for low-fat products. Although meat cuts have become leaner, the same cannot be said about meat products, which still have high levels of fat (<10 up to 50%) (JIMÉNEZ-COLMENERO, 2000). Burgers are frequently eaten products, mainly due to the current increase in the number of fast foods and because they are easy and fast to prepare (RODRÍGUEZ-CARPENA; MORCUENDE; ESTÉVEZ, 2012). Thus, due to its high fat content and popularity, beef burger is an attractive choice for fat reduction.

Fat presents important role in meat products, affecting sensory (juiciness, texture and flavor) (JIMÉNEZ-COLMENERO, 2000) and technological aspects (cooking loss, emulsion stability, water holding capacity, rheological properties) (HUGHES; COFRADES; TROY, 1997). Considering these aspects, the simple fat reduction raises a number of technological problems and leads to a decline in palatability, resulting in less accepted products (JIMÉNEZ-COLMENERO, 2000). Due to this, studies have been performed to reduce the fat amount of

burger and maintain its quality through the incorporation of non-meat ingredients. Some of these ingredients are fibers and vegetable oils.

Fiber is one of the most common functional ingredients used in food products (SÁNCHEZ-ZAPATA et al., 2010). Its importance in the food market is related to the wide range of properties, which go from physiological effects to technological properties. The insoluble dietary fiber acts as a bulking agent, normalizing intestinal motility, preventing constipation while soluble fiber is associated to decreasing the intestinal absorption of cholesterol and glucose (SILVEIRA RODRÍGUEZ; MONEREO MEGÍAS; MOLINA BAENA, 2003). Furthermore, fibers have been successfully applied in improving the water holding capacity (WHC) and oil holding capacity (OHC). These characteristics can be useful in products that require hydration, to avoid syneresis, improve yield, stabilize emulsions, and to modify texture and viscosity (ELLEUCH et al., 2011).

Traditionally, the fiber used as functional ingredient is obtained from cereals. However, according to recent studies, fruits and vegetable byproducts still contain high levels of dietary fiber (FERNÁNDEZ-LÓPEZ et al., 2008) with the advantage of having considerable amounts of antioxidants. The application of byproducts has already been studied in meat products, such as lemon albedo (ALESON-CARBONELL et al., 2005), passion fruit byproduct (LÓPEZ-VARGAS et al., 2014), horchata byproduct (SÁNCHEZ-ZAPATA et al., 2010), hazelnut pellicle (TURHAN; SAGIR; SULE USTUN, 2005). However, to the best of our knowledge, there is lack of studies about the application of pineapple byproduct in meat products.

With a world production exceeding 24 million tons in 2013 (FAO, 2013), pineapple is a widely consumed tropical fruit. Besides the fresh fruit, most of its production is used for the manufacture of several products, such as juices, fruit salads, canned fruits and jams. According to Larrauri, Rupérez, and Calixto (1997), residues from pineapple processing represent 25-35% of the fruit, and they still have bioactive compounds. Martínez et al. (2012), studying pineapple byproducts, reported high fiber content (75.8% dry basis) with 75.2% and 0.6% corresponding to insoluble and soluble fiber, respectively, as well as high water holding capacity and swelling capacity.

Vegetable oils are cholesterol free and they have higher proportion of unsaturated/saturated fatty acids compared to animal fats (CHOI et al., 2009). Canola oil presents good lipid profile, having the lowest levels of saturated fatty acids (SFAs) (7.36%) compared with other vegetable oils, such as sunflower (10.30%), corn (12.94%), olive (13.80%), soybean (15.65%), and cottonseed (25.9%) oils, high levels of monounsaturated

fatty acids (MUFAs) (63.27%), and intermediate levels of polyunsaturated fatty acids (PUFAs) (28.14%) (UNITED STATES, 2011). Its lipid composition has motivated studies based on its application in meat products as an alternative to minimize changes of the animal fat reduction, and also provide positive effects on consumer's health (SINGH et al., 2014).

Thus, the aim of this study was to evaluate the effect of pineapple byproduct and canola oil as fat replacers on cooking properties and chemical, physicochemical, texture and sensory characteristics of low-fat beef burgers.

4.2 Material and Methods

4.2.1 Byproduct preparation

Pineapple byproduct (a mixture of peel and pomace) was obtained from a commercial facility located in Jundiaí, SP, Brazil that produces more than one hundred different products, such as frozen fruits and vegetables, juices and pulps. At this facility, fruits were sanitized with 200 ppm of sodium hypochlorite, rinsed with water and then passed through the pulp extractor, where pineapple byproduct was collected. The sample of this byproduct collected for this research was kept frozen until its transportation to the Laboratory of Food and Nutrition of the “Luiz de Queiroz” College of Agriculture, University of São Paulo – ESALQ/USP (Piracicaba, SP, Brazil). Upon receiving sample was freeze dried (EC Modulyo, EC Apparatus Inc., New York, USA) during 96 h, ground using a knife mill (Marconi, Piracicaba, SP, Brazil), passed through a 40-mesh sieve (420 µm) and stored at -18 °C. Before the burger processing, pineapple byproducts underwent a thermal treatment (100 °C, 2 h) in order to inactive the bromelain.

4.2.2 Burger manufacture

Fresh beef (moisture 77.24%, fat 1.29%) and back fat (moisture 12.06%, fat 84.09%) were obtained from the local market. Beef and fat were separately ground (Hobart 4B22-2, Troy, OH, USA) using a 0.8 cm plate and then beef was divided into 5 treatments. The first treatment was used as a conventional formulation (CN) and the fat content was adjusted to 20% by the addition of back fat. The second treatment was used as a low-fat control (CT) and the fat content was adjusted to 10%. For the other treatments, pineapple byproduct (1.5%) and/or canola oil emulsion (5%) were used and the fat content was also adjusted to 10% (Table 1). The concentration of pineapple byproduct was selected based on a previous experiment that evaluated different concentrations of pineapple byproduct (1.0, 1.5, 2.0, 2.5%) and canola oil (5%) as fat substitutes in low-fat beef burger (chapter 3) (SELANI et al.,

2015). Canola oil emulsion was prepared by mixing (Ultra Turrax Ika T18 basic, Wilmington, NC, USA) for 2 min, at 10.000 rpm, eight parts of mineral hot water (50-55 °C) with one part of isolated soy protein and then with 10 parts of canola oil for other 3 min, at 10.000 rpm (MUGUERZA et al., 2001).

Table 1 - Formulations of beef burgers with addition of canola oil and pineapple byproduct

Ingredients	Treatments (%)				
	CN	CT	PA	CO	PC
Beef meat	70	70	70	70	70
Back fat	20	10	10	10	10
Cold water	7.5	17.5	16	12.5	11
Canola oil emulsion	0	0	0	5	5
Pineapple byproduct	0	0	1.5	0	1.5
Salt	1.5	1.5	1.5	1.5	1.5
Mix for burger*	1	1	1	1	1

*Commercial mix for burger: salt, maltodextrin, sodium polyphosphate, sodium erythorbate, natural spices and monosodium glutamate.

CN: conventional, with 20% fat; CT: control, with 10% fat; PA: with 10% fat and 1.5% of pineapple byproduct; CO: with 10% fat and 5% of canola oil; PC: with 10% fat, 1.5% of pineapple byproduct and 5% of canola oil.

After the addition of the meat, fat, pineapple byproduct and canola oil emulsion, the treatments were mixed with salt (1.5%), a commercial mix for burger (salt, maltodextrin, sodium polyphosphate, sodium erythorbate, natural spices and monosodium glutamate) (IBRAC, Rio Claro, SP, Brazil) and cold water. The formulations were kneaded by hand for 5 min and then 100 g portions were manually shaped using a burger-maker, to give the dimensions of 10 cm diameter and 1 cm thickness. The burgers were then placed in polyethylene packages and stored under -18 °C until further analyses. Part of the analyses was performed in both raw and cooked burgers. The cooking procedure occurred before these analyses, in a hot plate (150 °C) (Edanca, São Bernardo do Campo, SP, Brazil), until a meat core temperature of 75 °C. The processing occurred in triplicate (three independent burger processing).

4.2.3 Proximate composition

Moisture, ash, lipid (Soxhlet), and protein (Kjeldahl, N x 6.25) were determined in cooked burgers, in triplicate, according to Association of Official Analytical Chemists – AOAC (2010). Carbohydrates were calculated by difference.

4.2.4 Physicochemical analysis

4.2.4.1 pH

The pH was determined using a potentiometer (Oakton pH 300 series 35618, Vernon Hills, IL, USA) with automatic temperature compensation and glass penetration electrode (Digimed, Presidente Prudente, SP Brazil). The analysis was performed on five raw and cooked samples of each treatment, with three readings in each sample.

4.2.4.2 Color evaluation

The color of raw and cooked burgers was determined using a colorimeter (Konica Minolta, Chroma Meter, CR-400, Mahwah, NJ, USA) with a measurement area of 8 mm diameter, observation angle of 10° and illuminant C. The parameters were calibrated in standard white porcelain with $Y=93.7$, $x=0.3160$ and $y=0.3323$ and the following parameters were determined: lightness (L^*), redness (a^*), and yellowness (b^*). Hue (H^*) and chroma (C^*) were calculated according to the Eq. 1 and 2:

$$Hue = \tan^{-1} \cdot \frac{b^*}{a^*} \quad (1)$$

$$Chroma = (a^{*2} + b^{*2})^{1/2} \quad (2)$$

To determine the color, a surface layer was removed from both sides of the product. The analysis was performed on five samples of each treatment, with three readings in each sample.

4.2.4.3 Water activity

Water activity (A_w) was measured in raw and cooked burgers at 25 °C using the analyzer Aqualab CX 2T (Decagon Devices Inc., Pullman, WA, USA). The analysis was performed in triplicate.

4.2.5 Cooking properties

Three beef burgers of each treatment were cooked according to the procedures previously described and then samples were cooled to room temperature (25 °C) before weighing.

Cooking loss (CL) was calculated as weight loss divided by original weight, expressed as percentage. Moisture retention (Eq. 3), fat retention (Eq. 4), and diameter reduction (Eq. 5) were obtained according to the described by Sánchez-Zapata et al. (2010):

$$\% \text{ Moisture retention} = \frac{\text{Cooked weight} \cdot \% \text{ Moisture in cooked burger}}{\text{Raw weight} \cdot \% \text{ Moisture in raw burger}} * 100 \quad (3)$$

$$\% \text{ Fat retention} = \frac{\text{Cooked weight} \cdot \% \text{ Fat in cooked burger}}{\text{Raw weight} \cdot \% \text{ Fat in raw burger}} * 100 \quad (4)$$

$$\% \text{ Diameter reduction} = \frac{\text{Raw diameter} - \text{Cooked diameter}}{\text{Raw diameter}} * 100 \quad (5)$$

4.2.6 Texture analysis

Texture profile analysis (TPA) and Warner-Bratzler shear force (WB) were determined using a Texture Analyzer TA-XT (Stable Micro Systems, Godalming, United Kingdom). Prior to the analyses, samples were cooked according to the procedures previously described and cooled. Both analyses were performed with samples at 25° C.

For TPA, four burgers of each treatment were used and from each burger four cylindrical samples (2.5 cm) were taken. Then, samples were subjected to a two-cycle compression test, using a cylindrical probe of 3.5 cm diameter (P/35, Stable Micro Systems, Godalming, United Kingdom). Samples were compressed to 75% of their original height at a constant speed of 20 cm/min (pre-test speed and post-test speed: 40 cm/min). Hardness (N), springiness, cohesiveness, and chewiness were determined as described by Bourne (1978).

For WB, two 2.5 cm wide x 5.0 cm long strips were removed per burger, and five burgers were used from each treatment. The Warner Bratzler blade set (HDP/WBV, Stable Micro Systems, Godalming, United Kingdom) was used and the crosshead speed of the test was 25 cm/min. The instrumental value obtained was the peak force (N).

4.2.7 Microbiological analyses

Microbiological analyses were performed after the samples were cooked to verify the hygienic quality of the sample processing according to the limits specified by the RDC n° 12, of the “Agência Nacional de Vigilância Sanitária” (BRASIL, 2001). Microbial counts were

determined by diluting 25 g of sample in 225 mL of sterile peptone water (PW) (Difco Laboratories, Detroit, MI, USA) for *Escherichia coli*, thermotolerant coliforms, coagulase-positive staphylococci, and sulfite-reducing clostridia and in 225 mL of lactose broth (Difco Laboratories, Detroit, MI, USA) for *Salmonella*. Following dilution, samples were blended using a Stomacher (Seward Laboratory Systems, Bohemia, NY, USA), for 1 min. Further serial dilutions were prepared for microbial determinations.

Salmonella testing was performed by a pre-enrichment with lactose broth and then enriched samples were applied to the 1-2 test for *Salmonella* (BioControl Systems Inc., Bothell, WA, USA; recognized as an AOAC official method), according to the manufacturer's directions.

Escherichia coli and thermotolerant coliforms were counted using the multiple-tube fermentation test and expressed as most probable number (MPN)/g sample. Coagulase-positive staphylococci were counted by inoculating samples onto the surface of sterile plates with Baird-Parker agar (Difco Laboratories, Detroit, MI, USA). Sulfite-reducing clostridia were counted by inoculating samples on sterile plates with sulfite-polymyxin-sulfadiazine agar (Himedia, Mumbai, India), overlaid with 5 mL of the same medium and incubated in plastic anaerobic jars (2.5 L) using AnaeroGen sachet (Oxoid, Hampshire, United Kingdom). The determinations were performed in duplicate and the counts expressed as colony forming unit (CFU)/g sample. These analyses were performed according to the described by Downes and Ito (2001).

4.2.8 Quantitative descriptive analysis (QDA)

The quantitative descriptive analysis of the cooked burgers was approved by the Ethics Committee of Human Research of the ESALQ/USP (Piracicaba, SP, Brazil, protocol n° 92) and developed in nine 2-hours sessions, according to the method developed by Stone (1992). The QDA was determined by eight panelists, who were recruited from the students and staff of the Department of Agri-food Industry, Food and Nutrition, ESALQ/USP (Piracicaba, SP, Brazil). In the first stage of the analysis, seventeen candidates agreed to participate, and a pre-selection was performed to evaluate their ability to discriminate tastes and odors through basic taste tests, odor recognition tests and sequential analysis using triangle tests. For the next stage thirteen panelists were selected.

For the descriptive terminology development, Kelly's Repertory Grid Method (MOSKOWITZ, 1983) was used. All samples were presented in pairs, and the panelists described the similarities and differences between them for the attributes appearance, odor,

flavor, and texture. A list of terms was obtained and through an open discussion with a panel leader, the panelists had to evaluate all of them and exclude the redundant terms. After that, the panel established, by consensus, the definition of each attribute and its reference material (Table 2). In another session, all the references were presented to the panelists in order to attain a better understanding and a final agreement on how to measure all the attributes.

Table 2 - Attributes, definitions and scale extremes used in the QDA of beef burgers

Parameter	Attribute	Definitions	Scale extremes
External appearance	Brown color	Characteristic color of burger, ranging from light to dark brown	Light: chicken burger cooked until internal temperature of 75 °C Dark: beef burger cooked until internal temperature of 90 °C
Internal appearance	Cohesiveness	Uniform and compact appearance	Slight: cooked ground beef A lot: chicken sausage
Odor	Beef burger	Characteristic of commercial beef burger, made with beef, fat and seasonings	None: water Strong: Commercial beef burger cooked until internal temperature of 75 °C
Texture	Tenderness	Force needed to obtain deformation and cutting of the product by compression between teeth	Slight: <i>Biceps femoris</i> steak, cooked until internal temperature of 90 °C A lot: <i>Psoas major</i> steak, cooked until internal temperature of 70 °C
	Springiness	Ability of the product to return to its original shape, after compression between teeth	Slight: hard candy A lot: gummy bears
	Juiciness	Amount of moisture/ liquid released by the first bites	Slight: <i>Semitendinosus</i> steaks, cooked until internal temperature of 90 °C A lot: <i>Psoas major</i> steak, cooked until internal temperature of 70 °C
Flavor	Beef burger	Characteristics of commercial beef burger, made with beef, fat and seasonings	None: water Strong: Commercial beef burger cooked until internal temperature of 75 °C

Different training sessions were conducted until the panel presents repeatability, agreement among the members and ability to discriminate samples. In this stage, eight

panelists went to the final assessment of the QDA. This number of panelists is in accordance to the reported by Lawless and Heymann (2010) and Heymann et al. (2012), which indicate that the required number of panelists for obtaining stable results in the QDA is between 8 and 12.

For the formal assessment, burgers were cooked as previously described. Samples were cut into cubes (1.5 cm x 1.5 cm x 1.5 cm), random coded with three-digit numbers and presented in a sequential monadic way, following a balanced complete block design (MACFIE et al., 1989). The evaluations were conducted in individual booths equipped with lighting fluorescent lamps using a non-structured 10 cm-long scale, which was anchored as light and dark (brown color), slight and a lot (cohesiveness, tenderness, springiness, juiciness) and none and intense (odor and flavor of beef burger). After training, formal assessment was carried out in duplicate (two sessions).

4.2.9 Experimental design and Statistical analysis

The study was a randomized block design, with three blocks (each block corresponding to an independent burger processing). An analysis of variance (ANOVA) was carried out to analyze the results and the comparisons of treatments were performed by the Tukey's test ($P < 0.05$), using the software SAS (version 9, SAS Institute Inc., North Carolina, USA).

For the QDA, a three-factor ANOVA for each descriptor was carried out on the trained assessor scores, considering sample, session, assessor and their interaction as sources of variation. Tukey's test ($P < 0.05$) was used to compare the treatments. QDA results were also evaluated by principal component analysis (PCA) in a correlation matrix with the data centered and scaled on the mean. This analysis was performed to study the relationship among the intensity of the evaluated attributes and to get a sample map based on the intensity of the sensory attributes. Sensory data were performed with the software R, using SensoMineR (LE; HUSSON, 2008) and XLSTAT (Addinsoft, New York, USA).

4.3 Results and Discussion

4.3.1 Proximate composition

Differences in moisture contents were mainly related to the amount of water added to the formulations. However, for the two treatments with pineapple byproduct, which presented the highest values (Table 3), the increment in moisture was probably related to the presence of fiber in the product, providing higher water retention to the burgers. Similar result was found

by Choi et al. (2009) studying the addition of rice bran fiber and vegetable oils in meat batters.

Table 3 - Proximate composition (g/100g) of cooked burgers with addition of fruit byproducts and canola oil

Treatment	Moisture	Protein	Fat	Ash	Carbohydrates
CN	53.50±2.09 ^c	22.89±1.32 ^a	16.28±2.03 ^a	4.33±0.32 ^a	3.00±1.18 ^a
CT	58.09±1.28 ^{ab}	22.33±0.90 ^a	12.27±1.37 ^b	4.14±0.42 ^{ab}	3.16±0.73 ^a
PA	61.80±1.62 ^a	18.49±0.95 ^b	11.98±0.17 ^b	3.84±0.11 ^b	2.86±0.69 ^a
CO	56.95±1.86 ^{bc}	22.83±0.84 ^a	12.53±1.13 ^b	4.23±0.11 ^{ab}	3.46±1.12 ^a
PC	58.74±2.30 ^{ab}	19.73±1.40 ^b	12.93±1.04 ^b	4.11±0.21 ^{ab}	3.46±0.74 ^a

Carbohydrates by difference.

Mean ± Standard deviation.

Different letters in the same column differ significantly ($P<0.05$) by the Tukey's test.

CN: conventional, with 20% fat; CT: control, with 10% fat; PA: with 10% fat and 1.5% of pineapple byproduct; CO: with 10% fat and 5% of canola oil; PC: with 10% fat, 1.5% of pineapple byproduct and 5% of canola oil.

Protein content was significantly lower for PA and PC, which could be attributed to dilution effects resulting from pineapple byproduct addition to the formulations. Same effects were reported by López-Vargas et al. (2014) studying passion fruit albedo and by Sánchez-Zapata et al. (2010) with tiger nut fiber. As expected, conventional burgers had significantly higher fat content compared with low-fat treatments. The differences between the results and the target fat levels (20% for CN, 10% for the low-fat treatments) were probably due to cooking effects: excessive fat loss in CN (low fat retention, Table 5) that can be a result of the saturation of the protein:fat binding (difficulty of the protein matrix to hold the high amount of fat); and concentration of the compounds with the water loss in the other treatments. Ash content ranged from 3.84 to 4.33 and it was lower in the low-fat treatments probably because part of the fat was replaced by water. Different results were found by previous studies, which reported an increase in ash content when byproducts rich in fiber were added to burgers (SÁNCHEZ-ZAPATA et al., 2010; LÓPEZ-VARGAS et al., 2014). Despite the addition of pineapple byproduct, carbohydrate levels were not affected by any of the formulations.

4.3.2 Physicochemical analysis

The differences in lightness (L^*), redness (a^*), yellowness (b^*), chroma (C^*) and hue angle (H^*) of raw burgers were significant (Table 4). CN and CO treatments resulted in lighter burgers, with L^* values of 49.10 and 47.19, respectively.

Since conventional formulation had 10% more fat than low-fat treatments, it was expected that the color of its final product would be lighter. The addition of canola oil

increased lightness of both CO and PC, however just CO presented values similar to the conventional. This effect can be explained by the study of Youssef and Barbut (2011), who reported that the increase in lightness of meat emulsion with canola oil was probably related to the much smaller canola oil globules, which reflect more light (larger surface area) than the larger animal fat globules. Similar results were found by Rodríguez-Carpena et al. (2012), studying the addition of avocado, sunflower, and olive oils in pork burgers. PA and PC were darker than the conventional burgers and they showed decrease in redness compared to all of the other treatments, indicating that the color of the pineapple byproduct affected the characteristic color of the burgers. Darkening of samples was found by Choi et al. (2012), with the addition of seaweed (*L. japonica*) in reduced-fat pork burger and decrease in redness was reported by Sánchez-Zapata et al. (2010) in pork burger with tiger nut fiber.

Yellowness was not significantly affected by the addition of canola oil and pineapple byproduct. Both treatments with pineapple byproduct had lower chroma (C^*) values compared to CN, indicating a decrease in color intensity. Regarding hue angle (H^*), PA and PC presented the highest values and were not significantly different from the conventional burgers, indicating that these treatments were closer to the yellow axis compared to CT. These results could be due to the fat color (light yellow) in CN and the influence of the pineapple byproduct color ($b^* = 20.65$, chapter 2) in PA and PC. Increase in C^* values of pork burgers with tiger nut fiber was found by Sánchez-Zapata et al. (2010), corroborating with this study.

Table 4 - Physicochemical properties of raw and cooked burgers with addition of fruit byproducts and canola oil

	Lightness (L*)	Redness (a*)	Yellowness (b*)	Chroma (C*)	Hue (H*)	pH	Aw
Raw Burger							
CN	49.10±5.39 ^a	23.99±1.30 ^a	14.10±1.08 ^a	27.84±1.15 ^a	30.47±2.51 ^{abc}	6.20±0.03 ^a	0.97±0.00 ^a
CT	43.43±3.63 ^{bc}	23.30±2.19 ^a	12.74±0.91 ^a	26.56±2.29 ^{ab}	28.70±1.32 ^c	6.17±0.08 ^a	0.97±0.00 ^a
PA	42.84±4.02 ^c	19.50±2.44 ^b	12.82±1.43 ^a	23.37±2.35 ^c	33.43±3.88 ^{ab}	5.97±0.08 ^b	0.97±0.00 ^a
CO	47.19±3.71 ^{ab}	24.20±3.30 ^a	14.12±2.11 ^a	28.03±3.86 ^a	30.25±1.35 ^{bc}	6.18±0.10 ^a	0.97±0.00 ^a
PC	44.84±3.56 ^{bc}	19.84±2.08 ^b	13.45±1.50 ^a	23.97±2.44 ^{bc}	34.15±1.84 ^a	6.01±0.08 ^b	0.97±0.00 ^a
Cooked Burger							
CN	45.96±0.99 ^a	9.94±0.93 ^{ab}	9.49±1.18 ^a	13.79±0.92 ^a	44.52±3.77 ^a	6.78±0.17 ^a	0.96±0.00 ^a
CT	46.21±1.06 ^a	10.28±0.27 ^a	9.69±0.21 ^a	14.27±0.37 ^a	44.32±2.10 ^a	6.75±0.15 ^a	0.96±0.00 ^a
PA	48.52±2.76 ^a	8.97±0.54 ^b	10.94±0.36 ^a	14.21±0.19 ^a	50.34±3.01 ^a	6.52±0.10 ^b	0.96±0.00 ^a
CO	45.09±2.67 ^a	9.97±0.16 ^{ab}	10.09±1.41 ^a	14.42±0.52 ^a	46.38±2.58 ^a	6.76±0.17 ^a	0.96±0.00 ^a
PC	46.68±3.54 ^a	9.13±0.82 ^b	10.45±1.64 ^a	14.25±0.44 ^a	49.38±5.58 ^a	6.54±0.14 ^b	0.96±0.00 ^a

Mean ± Standard deviation.

Different letters in the same column differ significantly ($P<0.05$) by the Tukey's test.

CN: conventional, with 20% fat; CT: control, with 10% fat; PA: with 10% fat and 1.5% of pineapple byproduct; CO: with 10% fat and 5% of canola oil; PC: with 10% fat, 1.5% of pineapple byproduct and 5% of canola oil.

After cooking, there were no significant differences among treatments for the L^* , b^* , C^* and H^* color parameters (Table 4). According to Sánchez-Zapata et al. (2010) the alterations caused by the cooking process (Maillard reaction, protein denaturation, and fat and water loss) could mask some undesirable color changes induced by formulation. This could explain the results of the present study, in which the fat reduction and the addition of pineapple byproduct and canola oil caused some color changes in raw samples, however in cooked samples no differences were found in lightness, yellowness, chroma and hue angle. Regarding a^* value, the three treatments with addition of fat substitutes (PA, CO, and PC) showed positive results, since their redness were similar to the conventional. The low-fat control (CT) had the highest a^* value probably because this treatment did not receive any additional ingredient to influence the color and due to its high cooking loss (Table 5), resulting in protein concentration, and consequently, higher redness.

The pH of raw and cooked samples presented the same tendency, showing that the incorporation of pineapple byproduct in burger significant decreased the pH values compared to those of the other treatments (Table 4). This pH reduction is certainly a result of the characteristics of the pineapple byproduct that has a pH of 4.08 and a titratable acidity 1.90 g citric acid/100 g (chapter 2). Similarly, López-Vargas et al. (2014) found a pH decrease in burgers with addition of passion fruit albedo. Even with fiber addition, no differences were observed in water activity of raw and cooked burgers (Table 4). This result is in agreement with the study of Aleson-Carbonell et al. (2005) with lemon albedo addition in beef burgers.

4.3.3 Cooking properties

PA and PC presented the lowest cooking loss (Table 5), and were significantly different from the other formulations. This is a result of the addition of the fruit byproduct rich in fiber (69.64 % dietary fiber, chapter 2) that presents the property to hold water and fat, reducing the fluid loss. Significantly lower cooking loss (or higher cooking yield) of burgers with fiber was also found in studies with wakame addition (brown seaweed, high in fiber) and olive oil (LÓPEZ-LÓPEZ et al., 2010) and with lemon albedo (ALESON-CARBONELL et al., 2005).

Table 5 - Cooking properties (%) of burgers with addition of pineapple byproduct and canola oil

Treatments	Cooking loss	Moisture retention	Fat retention	Diameter reduction
CN	39.15±2.04a	52.30±1.09bc	57.80±5.84b	27.58±2.25a
CT	41.33±0.61a	46.95±1.24c	85.20±7.14a	26.81±2.42a
PA	28.01±3.83b	63.39±4.98a	89.79±1.95a	18.79±4.95b
CO	40.79±1.10a	48.96±2.83c	68.24±5.91b	27.05±1.95a
PC	30.92±1.56b	59.52±3.82ab	82.41±11.87a	20.45±4.12b

Mean ± Standard deviation.

Different letters in the same column differ significantly ($P<0.05$) by the Tukey's test.

CN: conventional, with 20% fat; CT: control, with 10% fat; PA: with 10% fat and 1.5% of pineapple byproduct; CO: with 10% fat and 5% of canola oil; PC: with 10% fat, 1.5% of pineapple byproduct and 5% of canola oil.

Canola oil addition did not seem to interfere in cooking loss, since there was no significant difference between CO and the conventional burger. Different results were found by Dzudie et al. (2004), who reported higher cooking loss for beef patties formulated with ground-nut and maize oils (20%, not emulsified) in comparison with animal fat treatments (20%) and by Youssef and Barbut (2011) who found that meat emulsions with canola oil showed lower fluid losses compared to the corresponding beef fat treatments. Differences between studies may be due to the amount of animal fat replaced, percentage and type of vegetable oil studied, as well as the method of vegetable oil incorporation in the product.

As observed for cooking loss, PA and PC presented the highest values of moisture and fat retention and no differences were observed among conventional and canola oil added burgers (Table 5). The results are probably related to the functional properties of the fibers present in the pineapple byproduct (WHC: 4.96 g water/ g freeze-dried sample, OHC: 1.85 g oil/g freeze-dried sample, chapter 2), which avoided the excessive release of these components during cooking. The results are consistent with those reported by Sánchez-Zapata et al. (2010) studying horchata byproduct and by Aleson-Carbonell et al. (2005) with lemon albedo. Furthermore, differences of fat retention could also have been caused by the capacity of the protein matrix to retain lipids. The lower fat retention in the CN was probably due to its higher fat content, resulting in insufficient amount of meat proteins to hold all the fat added. For CO, the extra amount of fat provided by the canola oil addition may have caused the low fat retention.

The diameter reduction is the result of the denaturation of meat proteins with the loss of water and fat (BESBES et al., 2008). Since PA and PC showed the highest values of moisture and fat retention, it was expected that both treatments would present lower diameter reduction. Treatments without fiber addition reduced almost 8% more the burger diameter

compared to the treatments with pineapple byproduct (Table 5). Thus, the fruit byproduct rich in fiber promoted desirable effects, minimizing the common shape changes of comminuted products. Turhan, Sagir, and Ustun (2005) reported the same effect in burgers with addition of hazelnut pellicle.

4.3.4 Texture analysis

TPA hardness is defined as the maximum force required to compress the samples (BOURNE, 1978), while WB shear force is obtained by the maximum force required to shear them. Due to this, both WB shear force and TPA-hardness are usually used to predict tenderness in meats.

According to the TPA-hardness results, CN and CT burgers presented the lowest and the highest values, respectively. Since fat provides flavor, tenderness and juiciness to food products (KEETON, 1994), its reduction in the burgers studied here led to the development of harder products (Table 6). Furthermore, according to the study of Gao, Zhang, and Zhou (2014), hardness varied inversely with moisture retention of the meat product. Thus, since CT had the lowest moisture retention, this fact may also have contributed for obtaining harder products.

Table 6 - Textural properties and Warner Bratzler shear force of cooked burgers with addition of pineapple byproduct and canola oil

Treatments	Hardness (N)	Springiness	Cohesiveness	Chewiness	WB (N)
CN	129.45±4.11 ^b	0.67±0.04 ^a	0.32±0.02 ^b	2834.63±122.44 ^b	18.03±1.86 ^b
CT	208.09±7.85 ^a	0.75±0.05 ^a	0.42±0.02 ^a	6645.44±854.21 ^a	23.56±1.96 ^a
PA	185.37±5.67 ^a	0.74±0.04 ^a	0.41±0.03 ^a	5708.87±47.85 ^a	21.10±0.28 ^{ab}
CO	204.84±5.67 ^a	0.76±0.03 ^a	0.46±0.02 ^a	6841.82±905.22 ^a	21.86±1.60 ^a
PC	194.90±10.43 ^a	0.76±0.01 ^a	0.45±0.01 ^a	6705.92±252.36 ^a	21.19±0.25 ^{ab}

Mean ± Standard deviation.

Different letters in the same column differ significantly ($P<0.05$) by the Tukey's test.

WB: Warner Bratzler shear force.

CN: conventional, with 20% fat; CT: control, with 10% fat; PA: with 10% fat and 1.5% of pineapple byproduct; CO: with 10% fat and 5% of canola oil; PC: with 10% fat, 1.5% of pineapple byproduct and 5% of canola oil.

Treatments with fiber addition (PA and PC), even showing high moisture and fat retention (Table 5) and a slight decrease in hardness compared to the other low-fat formulations, were significantly harder than the conventional products. This result is in agreement with the study of Choi et al. (2009), who found that hardness of control meat batters (30% animal fat) was lower than those found in reduced-fat meat batters (10 % animal fat) containing vegetable oils and rice bran fiber. However, in another study of Choi et al.

(2012), they found a different result, with a decrease in hardness when fat was reduced and *Laminaria japonica* (DF: 51.23%) was added to pork patties. Since hardening and softening have been observed when fiber is added to meat products, these results may be due to the amount and characteristics of each type of fiber used, as well as the type of meat product studied.

On the other hand, for WB shear force, there was no significant difference among CN and the low-fat treatments with pineapple byproduct addition. The similar shear force values of PA and PC in relation to the conventional burger may have been caused by the presence of fiber in pineapple byproduct, which contributed to an increase in moisture and fat retention of the burgers, resulting in a tender product.

Regarding the effects of the canola oil addition, CO was significantly harder than CN in both methods. This result is in accordance to the study of Youssef and Barbut (2011), who found an increase in hardness of cooked meat batters with canola oil addition. According to Youssef and Barbut (2010), when canola oil is used, the fat globules formed are smaller than those obtained from animal fat. With a higher surface area, canola oil globules, covered by proteins, allows more bonding to the matrix, resulting in firmer products.

Despite the different results of TPA-hardness and WB shear force, mainly regarding pineapple byproduct addition, it was possible to observe that the fiber presence in beef burgers showed good results, minimizing the negative hardening of products with fat reduction.

Springiness was not affected by either the fat reduction or the canola oil and pineapple byproduct addition. Similar results were found in pork patties with rice flour (GAO; ZHANG; ZHOU, 2014) and in pork burgers with avocado, sunflower and olive oils (RODRÍGUEZ-CARPENA; MORCUENDE; ESTÉVEZ, 2012).

Conventional burger had the highest cohesiveness value and it was significant different from all the low-fat treatments. These treatments present lower fat:protein ratio, which may have led to higher amount of available proteins to form denser protein network (YOUSSEF; BARBUT; SMITH, 2011), resulting in the development of a more cohesive product. Similar result was found in low-fat meat emulsion with vegetable oils and rice bran fiber (CHOI et al., 2009).

For chewiness, there was no difference among CT, PA, CO, and PC, however they were all less chewy than conventional burgers. The highest chewiness of low-fat products is probably related to the fat reduction, and consequently, to the other texture parameters already discussed. Samples with higher hardness and cohesiveness probably need more work to

masticate them until swallowing. Higher chewiness of low-fat meat emulsion with sunflower seed oil and *makgeolli* lees fiber compared to control was reported in a previous study (CHOI et al., 2013).

4.3.5 Microbiological analyses

The microbial counts of all treatments were <2 MNP/g for thermotolerant coliforms and *E. coli*, <10 CFU/g for sulfite-reducing clostridia, $<10^2$ for coagulase-positive staphylococci, and there was absence of *Salmonella* in 25g. All treatments showed counts within the limits established by the RDC 12 of the “Agência Nacional de Vigilância Sanitária” (BRASIL, 2001): thermotolerant coliforms: 10^3 MPN/g, sulfite-reducing clostridia: 5×10^2 CFU/g, coagulase-positive staphylococci: 3×10^3 CFU/g, and *Salmonella*: absence/25 g. In spite of the highest moisture retention observed in PA and PC, microbial growth of the burgers was not affected by the addition of pineapple byproducts. According to the microbial counts, in the given experimental conditions, the burgers developed here were safe and fit for consumption from a microbiological standpoint.

4.3.6 Quantitative descriptive analysis

The results of the QDA showed significant effect for the attributes brown color, cohesiveness and juiciness.

PC and the CN showed similar intensity of brown color and the highest scores for this attribute, with values of 7.76 and 7.65, respectively (Table 7). Among the treatments with fat reduction, PA showed the lowest score, indicating that the addition of pineapple byproduct alone, due to its light color (lightness = 73.45, chapter 2), and high water holding capacity (4.96 g water/g dry sample, chapter 2), increasing moisture retention, resulted in burgers with a lighter brown color. The decrease in color intensity was also observed in pork burger with passion fruit albedo (LÓPEZ-VARGAS et al., 2014). Regarding CO scores, the low values may be explained by the relationship between the size of the fat globules and the light reflectance. According to Youssef, Barbut, and Smith (2011), the lighter appearance of products with canola oil is due to their smaller fat globules compared to those of animal fat.

Table 7 - Quantitative descriptive analysis scores of beef burgers with addition of pineapple byproduct and canola oil

Parameter	Attribute	Treatments				
		CN	CT	PA	CO	PC
External appearance	Brown color	7.65±0.85 ^a	6.05±1.52 ^b	5.71±1.16 ^b	6.32±1.19 ^b	7.76±0.81 ^a
Internal appearance	Cohesiveness	5.58±1.40 ^b	7.11±0.80 ^a	5.76±1.16 ^b	7.12±0.84 ^a	5.11±1.48 ^b
Odor	Beef burger	5.99±1.77 ^a	5.52±2.31 ^a	5.81±1.89 ^a	5.57±2.36 ^a	6.08±1.87 ^a
Texture	Tenderness	7.49±0.98 ^a	6.72±1.21 ^a	6.90±1.53 ^a	7.31±1.54 ^a	7.49±1.07 ^a
	Springiness	6.33±1.28 ^a	6.37±0.96 ^a	6.03±1.05 ^a	5.92±1.64 ^a	5.49±1.82 ^a
	Juiciness	7.39±0.70 ^a	6.11±1.27 ^b	6.41±1.20 ^{ab}	7.21±0.82 ^a	6.84±0.98 ^a
Flavor	Beef burger	6.70±1.06 ^a	6.37±1.64 ^a	6.01±1.62 ^a	6.29±1.81 ^a	6.33±1.39 ^a

n = 8

Mean ± Standard deviation.

Different letters in the same column differ significantly ($P < 0.05$) by the Tukey's test.

CN: conventional, with 20% fat; CT: control, with 10% fat; PA: with 10% fat and 1.5% of pineapple byproduct; CO: with 10% fat and 5% of canola oil; PC: with 10% fat, 1.5% of pineapple byproduct and 5% of canola oil.

There was a significant increase in cohesiveness with the fat reduction; however the addition of pineapple byproduct (PA and PC) positively affected the products, resulting in burgers with similar scores to CN. Youssef and Barbut (2011) reported that cohesiveness tends to decrease as fat content increase and in another study Youssef, Barbut, and Smith. (2011) found that high values of cohesiveness could be related to high cooking loss, leading to protein concentration, and the development of a more cohesive protein matrix. This may explain the results of the present study, since CN had 10% more fat than the other treatments, and among the low-fat treatments, PA and PC showed high fat retention and the lowest values of cooking loss.

Regarding juiciness, conventional burger had the highest score and it was not significantly different from PA, CO and PC. These results showed that fiber from pineapple byproduct and/or canola oil addition improved juiciness of low-fat beef burgers. The high juiciness of PA and PC indicated that the fiber from pineapple byproduct retained the appropriate amount of moisture and fat to assure a juicy product. In treatments with canola oil (CO and PC), since fat provides juiciness to food products, the addition of another source of fat improved this sensory aspect in the products. The results also showed that CT presented the lowest value, indicating that the simple fat reduction, without adding any fat substitute, negatively affected the meat product. Choi et al. (2012), reported that juiciness scores of reduced-fat pork patties containing 1% and 3% *L. japonica* powder (fiber= 51.23%) were significantly higher than the control, and that reduced-fat pork patties without *L. japonica* showed the lowest value.

Other positive aspect observed in the QDA was the fact that tenderness, springiness, odor, and flavor of the burgers were not affected by pineapple byproduct and canola oil addition.

A PCA was performed and according to the results, the sum of the principal components 1 and 2 (PC1 and PC2) accounted for 83.66% of the data variation.

PC1 explained the majority of the variations (59.64%) and as expected, conventional product was positioned on the opposite side of the low-fat control. CN and PC were located on the right side, indicating that they had higher intensity of brown color, tenderness, juiciness, odor and flavor of beef burger (Figure 1). These are important results, showing that the addition of fiber rich pineapple byproduct associated with canola oil positively influenced juiciness and tenderness, two of the most affected sensory parameters in products with fat reduction. Furthermore, the use of these two ingredients did not affect the odor and flavor of the burger. Cohesiveness and springiness, attributes negatively correlated to the PC 1, were higher in CT, CO and PA. This is in accordance to Keeton (1994), who described that as fat content of meat products declines, cohesiveness and springiness increase.

Figure 1 - Principal component analysis (PCA) of the sensory attributes of the beef burgers

PC2 explained 24.02% of the variance and was positively correlated with all the texture and appearance attributes, as well as the flavor of beef burger, presenting CN, CT, and

CO located at the top part of the graphic. The negative values were observed for odor of beef burger, indicating that PA and PC presented higher intensity of this attribute.

4.4 Conclusions

The use of pineapple byproduct was found to be effective in improving the cooking characteristics of the product, affecting positively the yield and appearance of low-fat burgers. Color of cooked burgers with addition of canola oil and/or pineapple byproduct was not affected, which is a significant result considering the importance of this attribute to the consumer's acceptability. In spite of the texture alterations observed in all the low-fat treatments (they were harder, chewier, and more cohesive than CN), the ones with pineapple byproduct addition (PA and PC) stood out, since according to the WB shear force results, they were as tender as the full-fat burger. QDA analysis showed that PC was not different from the conventional burger in any of the attributes evaluated, indicating that the use of the two fat replacers together can be a suitable choice to minimize the sensory alterations of the fat reduction in beef burgers.

The results of this study highlight the application of pineapple byproduct associated with canola oil emulsion as promising fat replacers in beef burger, opening the possibility of a new application for this fruit byproduct.

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5 PINEAPPLE BYPRODUCT AND CANOLA OIL AS PARTIAL FAT REPLACERS IN LOW-FAT BEEF BURGER: EFFECTS ON OXIDATIVE STABILITY, CHOLESTEROL CONTENT AND LIPID PROFILE

Abstract

The effect of pineapple byproduct and canola oil as fat replacers on the oxidative stability, cholesterol and fatty acid profile of low-fat beef burgers was evaluated. Five treatments were performed: conventional (CN, 20% fat) and four low-fat formulations (10% fat): control (CT), with pineapple byproduct (PA), with canola oil (CO), and with pineapple byproduct and canola oil (PC). Low-fat cooked burgers showed a mean cholesterol reduction of 9.15% compared to the CN. Canola oil addition improved the lipid profile of the burgers, which was observed through an increase in the polyunsaturated/saturated fatty acids ratio and a decrease in the n-6/n-3 ratio, in the atherogenic and thrombogenic indexes. The oxidative stability of the burgers was affected by the vegetable oil addition. However, at the end of the storage time, lipid oxidation of CO and PC was lower than the threshold for the consumer's acceptance. Canola oil, in combination with pineapple byproduct, can be considered promising fat replacers in the development of healthier burgers.

Keywords: Lipid oxidation; Fatty acid profile; Vegetable oil; Fruit byproduct; Fat substitutes

5.1 Introduction

Burger is one of the most popular processed meat products in the world. It is highly accepted and consumed, mainly due to the current increase in the number of fast foods worldwide and due to its convenience and low price (HOOGENKAMP, 1997). However, burgers are also known by some negative aspects, such as the quantity (20-30%) (JIMÉNEZ-COLMENERO, 2000) and quality of its fat (mostly saturated fatty acids), as well as the cholesterol content, which are associated with the occurrence of some chronic and cardiovascular diseases (FERNÁNDEZ-GINÉS et al., 2005).

With the increased concerns about the relationship between fat intake and health, consumers have become more conscious regarding a healthy diet, demanding products with reduced fat, cholesterol, and altered fatty acid profile (OSPINA et al., 2012). Thus, due to the high fat content and popularity of the burgers, they are considered an attractive choice for fat reduction and lipid profile improvement.

However, the fat reduction of meat products, with its direct substitution by water, can bring a series of deleterious effects in both sensory quality (reducing flavor and juiciness, and modifying texture) (JIMÉNEZ-COLMENERO, 2000) and technological characteristics (increasing cooking loss, reducing yield, affecting emulsion stability) (HUGHES; COFRADES; TROY, 1997). In order to minimize these issues and to improve the quality of

reduced-fat products, some ingredients have been studied to act as animal fat replacers, such as dietary fibers (DF) and vegetable oils.

The use of dietary fibers as a functional ingredient is related to their interesting properties that can positively affect some technological characteristics of the meat products. Fibers have been successfully applied to improve water holding capacity (WHC), oil holding capacity (OHC) and swelling capacity, which are useful in products that require hydration, to avoid syneresis, improve yield, stabilize emulsions, and modify texture and viscosity (ELLEUCH et al., 2011). Furthermore, it is well-known that DF plays an important role in human health, acting as a bulking agent, normalizing intestinal motility and then preventing constipation (insoluble fibers) and decreasing the intestinal absorption of cholesterol and glucose (soluble fibers) (SILVEIRA RODRÍGUEZ; MONEREO MEGÍAS; MOLINA BAENA, 2003).

Dietary fibers are obtained mainly from cereals. However, fruits and vegetable byproducts still have high DF content, with the advantage of presenting considerable amounts of antioxidants (DENG; PENNER; ZHAO, 2011; MARTÍNEZ et al., 2012). Pineapple is a widely consumed tropical fruit and part of its production is intended to the manufacture of juices, fruit salads, canned fruits and jams. The residues generated by this industrial activity are composed mainly by peel and core and represent about 25-35% of the fruit (LARRAURI; RUPÉREZ; CALIXTO, 1997). According to a previous study, pineapple byproduct (peel and heart) presents DF as its major component (75.8%), in addition to have high water holding capacity, swelling capacity and an interesting antioxidant activity (MARTÍNEZ et al., 2012).

Besides the dietary fiber, the replacement of animal fat with vegetable oil has been used as an alternative to improve technological and sensory aspects of low-fat meat products, in addition to enhance its nutritional value, by reducing saturated fatty acids (SFAs), cholesterol and increasing monounsaturated (MUFAs), and polyunsaturated fatty acids (PUFAs). Canola oil has an interesting lipid profile, showing the lowest level of SFAs (7.36%) among the most common vegetable oils, such as sunflower (10.30%), corn (12.94%), olive (13.80%), soybean (15.65%), and cottonseed (25.9%) oils, high levels of monounsaturated fatty acids (MUFAs) (63.27%), and intermediate levels of polyunsaturated fatty acids (PUFAs) (28.14%) (UNITED STATES, 2011). Its lipid composition has motivated some studies based on canola oil application in meat products, resulting in positive effects regarding technological (YOUSSEF; BARBUT, 2011) and nutritional characteristics (PELSER et al., 2007) of the products.

To the best of our knowledge, there is lack of studies about the application of pineapple byproduct in meat products and just a few studies evaluating the association of fiber and vegetable oils as fat replacers. In this context, the present work aimed to study the impact of animal fat reduction and its partial substitution by pineapple byproduct and canola oil on the oxidative stability, cholesterol content and fatty acid profile of beef burgers.

5.2 Material and Methods

5.2.1 Byproduct preparation

Pineapple byproduct (a mixture of peel and pomace) was obtained from a commercial facility located in Jundiaí, SP, Brazil that produces more than one hundred different products, such as frozen fruits and vegetables, juices and pulps. At this facility, fruits were sanitized with 200 ppm of sodium hypochlorite, rinsed with water and then passed through the pulp extractor, where pineapple byproduct was collected. The sample of this byproduct collected for this research was kept frozen until its transportation to the Laboratory of Food and Nutrition of the “Luiz de Queiroz” College of Agriculture, University of São Paulo – ESALQ/USP (Piracicaba, SP, Brazil). Upon receiving, samples were freeze dried (EC Modulyo, EC Apparatus Inc., New York, USA) during 96 h, ground using a knife mill (Marconi, Piracicaba, SP, Brazil), passed through a 40-mesh sieve (420 μ m) and stored at -18 °C. Before the burger processing, pineapple byproducts underwent a thermal treatment (100 °C, 2 h) in order to inactive the bromelain.

5.2.2 Burger manufacture

Fresh beef (moisture 77.24%, fat 1.29%) and back fat (moisture 12.06%, fat 84.09%) were purchased from a local slaughterhouse (Piracicaba, SP, Brazil). Beef and fat were separately ground (Hobart 4B22-2, Troy, OH, USA) using a 0.8 cm plate and then beef was divided into 5 treatments. The first treatment was used as a conventional formulation (CN) and the fat content was adjusted to 20% by the addition of back fat. The second treatment was used as a low-fat control (CT) and the fat content was adjusted to 10%. For the other treatments, pineapple byproduct (1.5%) and/or canola oil emulsion (5%) were used and the fat content was also adjusted to 10% (Table 1). The concentration of pineapple byproduct was selected based on a previous experiment that evaluated different concentrations of pineapple byproduct (1.0, 1.5, 2.0, 2.5%) and canola oil (5%) as fat substitutes in low-fat beef burger (chapter 3) (SELANI et al., 2015). Canola oil emulsion was prepared by mixing (Ultra Turrax Ika T18 basic, Wilmington, NC, USA) for 2 min, at 10.000 rpm, eight parts of mineral hot

water (50-55 °C) with one part of isolated soy protein and then with 10 parts of canola oil for other 3 min, at 10.000 rpm (MUGUERZA et al., 2001).

Table 1 - Formulations of beef burgers with addition of canola oil and pineapple byproduct

Ingredients	Treatments (%)				
	CN	CT	PA	CO	PC
Beef meat	70	70	70	70	70
Back fat	20	10	10	10	10
Cold water	7.5	17.5	16	12.5	11
Canola oil emulsion	0	0	0	5	5
Pineapple byproduct	0	0	1.5	0	1.5
Salt	1.5	1.5	1.5	1.5	1.5
Mix for burger*	1	1	1	1	1

* Commercial mix for burger: salt, maltodextrin, sodium polyphosphate, sodium erythorbate, natural spices and monosodium glutamate

CN: conventional, with 20% fat; CT: control, with 10% fat; PA: with 10% fat and 1.5% of pineapple byproduct; CO: with 10% fat and 5% of canola oil; PC: with 10% fat, 1.5% of pineapple byproduct and 5% of canola oil

After the addition of the respective amount of beef, fat, pineapple byproduct and canola oil emulsion, the treatments were mixed with salt (1.5%), a commercial mix for burger (salt, maltodextrin, sodium polyphosphate, sodium erythorbate, natural spices and monosodium glutamate) (IBRAC, Rio Claro, SP, Brazil) and cold water. The formulations were kneaded by hand for 5 min and from the homogenized meat mixture, 100 g portions were manually shaped using a burger-maker, to give the dimensions of 10 cm diameter and 1 cm thickness. The beef burgers were then placed in polyethylene packages and stored under - 18 °C for further analyses. The cooking procedure occurred before the analyses, in a hot plate (150 °C) (Edanca, São Bernardo do Campo, SP, Brazil), until a meat core temperature of 75 °C. The processing occurred in triplicate (all the formulations were applied to three independent batches of meat and fat).

5.2.3 Cholesterol

Cholesterol was extracted in triplicate, by direct saponification, according to the method described by Almeida et al. (2006). Two grams of the sample were saponified with 4 mL of a 50% aqueous solution of potassium hydroxide and 6 mL of ethanol. The samples were incubated in a shaking water bath (Solab model SL157, Piracicaba, SP, Brazil) at 40 °C until complete solubilization and then they were heated for 10 min at 60 °C. After this, 5 mL of distilled water were added and the samples were cooled in cold water. The non-saponifiable matter was extracted three times with 10 mL of hexane. The hexane extract was dried using a vacuum rotary evaporator (Fisatom model 801, São Paulo, SP, Brazil) and

nitrogen gas. After this, the sample was diluted with 1 mL of acetonitrile:isopropanol (85:15, v/v), filtered through a 0.45 μm PTFE membrane into autosampler vials and then injected (20 μL) in a high performance liquid chromatography (HPLC).

The HPLC system (Shimadzu LC-20AT, Kyoto, Japan) was equipped with a UV-visible detector (Shimadzu SPD-20A, Kyoto, Japan), an ODS column and its corresponding guard column (Zorbax Eclipse plus C18, 4.6 mm x 250 mm, 5 μm). The column was isocratically eluted at 40 °C with acetonitrile and isopropanol (85:15, v/v) at a flow rate of 2 mL/min and an analysis time of 15 min. The detector was set at 210 nm. Cholesterol quantification was done by external standardization, using a cholesterol authentic standard (Sigma Aldrich, St. Louis, MO, USA).

5.2.4 Fatty acid profile

Fat was extracted in triplicate according to the method of Bligh and Dyer (1959) and the fatty acid methyl esters (FAMES) were obtained according to the methodology described by Hartman and Lago (1973), with modifications. FAMES were analyzed using gas chromatography (GC) (Shimadzu, Model GC 2010, Kyoto, Japan) equipped with a fused-silica capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness) (Restek Stabilwax, PA, USA) and a flame ionization detector. Gas chromatograph oven program temperature was as follows: initial temperature of 180 °C for 3 min, then it was raised to 210 °C at a rate of 5 °C/min and kept at this temperature for 13 minutes, and finally the temperature was raised to 220 °C at a rate of 10 °C/min and kept at 220 °C for 7 min. The injector and detector temperatures were 180°C and 250°C, respectively. Carrier gas was nitrogen at a flow rate of 4 mL/min. The split ratio was 1:10. Samples (1 μL) were injected using an automatic injector (Shimadzu AOC-20i). Individual FAMES peaks were identified by comparison of their retention times with those of the standards (FAME Mix C8-C24, Supelco, USA). Quantification was performed using tridecanoic acid as internal standard. The results were expressed in grams per 100 g of detected FAMES and the analysis was carried out in triplicate.

Based on the FAME results, the atherogenic (AI) (Eq. 1) and thrombogenic indexes (TI) (Eq. 2) were calculated according to Ulbricht and Southgate (1991), where C12:0, C14:0, C16:0, C18:0, n – 6 PUFA, and n - 3 PUFA are lauric, miristic, palmitic, stearic, linoleic and linolenic acids, respectively.

$$AI = \frac{C12:0 + 4 * C14:0 + C16:0}{MUFA + n - 3 PUFA + n - 6 PUFA} \quad (1)$$

$$TI = \frac{C14:0 + C16:0 + C18:0}{0.5 * MUFA + 0.5 * n - 3 PUFA + 3 * n - 6 PUFA + \frac{n - 3 PUFA}{n - 6 PUFA}} \quad (2)$$

5.2.5 Lipid oxidation

Lipid oxidation was evaluated by the malonaldehyde (MDA) quantification. MDA was extracted in triplicate, through the method described by Vyncke (1970, 1975) and Sørensen and Jørgensen (1996), with modifications, and quantified using HPLC. For extraction, 5 g of meat was homogenized in Ultra Turrax (Ika T18 basic, NC, USA) at 10.000 rpm for 30 s with 15 mL of a 7.5% trichloroacetic acid, 0.1% propyl gallate, and 0.1% ethylene diamine tetraacetic acid aqueous solution. After filtration, 5 mL of the extract were mixed with 5 mL of a 0.02 M thiobarbituric acid aqueous solution in capped test tubes. The samples were incubated in a water bath (Solab SL157, Piracicaba, SP, Brazil) at 100 °C for 40 min and then cooled in cold water. After that, the samples were filtered through a 0.45 µm PTFE membrane into autosampler vials and injected (20µL) into a HPLC.

The HPLC system (Shimadzu LC-20AT, Kyoto, Japan) was equipped with a fluorescence detector (Shimadzu RF-20A, Kyoto, Japan), an ODS column and its corresponding guard column (Zorbax Eclipse plus C18, 4.6 mm x 250 mm, 5 µm). The column was isocratically eluted at 40 °C, with sodium phosphate buffer (pH 7.0, 5 mM):isopropanol (85:15, v/v) at a flow rate of 1 mL/min and an analysis time of 10 min. The fluorescence detector was set at 515 nm (excitation) and 543 (emission). A standard curve was performed with 1,1,3,3-tetraethoxypropane and the results were expressed as mg of MDA per kg of sample.

5.2.6 Experimental design and Statistical analysis

The study was a randomized block design, with three blocks (each block corresponding to an independent burger processing), except for the malonaldehyde content.

For malonaldehyde, a randomized block design with two blocks was considered, and for this analysis a 5 x 5 full factorial experiment was conducted, considering as factors the treatments (CN, CT, PA, CO, PC) and the storage times (0, 30, 60, 90, and 120 days).

Analysis of variance (ANOVA) was carried out to analyze the results and the comparisons of treatments and storage times were performed by the Tukey's test ($P<0.05$), using the software SAS (version 9, SAS Institute Inc., North Carolina, USA).

5.3 Results and Discussion

5.3.1 Cholesterol

The results of the raw samples showed that CN had a cholesterol content of 82.42 mg/kg while low-fat burgers had the cholesterol ranging from 73.33 to 76.75 mg/kg (Table 2). Despite this decrease, the cholesterol reduction was not significant. Similar to these findings, no differences in the cholesterol content of full-fat and low-fat treatments were observed in the study of Bond, Marchello, and Slinger (2001), who evaluated conventional (20% fat) and reduced-fat burgers (10% fat), and in the study of Rodríguez-Carpena, Morcuende, and Estévez (2012), comparing control (10% fat) and low-fat burgers (5%) with vegetable oils. On the other hand, Choi et al. (2010) reported significantly lower cholesterol in reduced-fat frankfurters with vegetable oil and rice bran fiber (10% fat) than the control (30% fat). The differences among studies are certainly related to the amount of fat removed from the formulation. Higher levels of fat reduction, as those observed in the study of Choi et al. (2010), probably led to higher impact on the cholesterol amount, and could have more reasonably caused its significant decrease.

Table 2 - Cholesterol (mg/100 g sample) of raw and cooked burgers with addition of pineapple byproduct and canola oil

Treatments	Cholesterol	
	Raw Burger	Cooked Burger
CN	82.42±13.20 ^a	117.42±12.64 ^a
CT	76.75±20.87 ^a	107.06±14.43 ^b
PA	74.70±16.89 ^a	105.31±15.41 ^b
CO	73.33±17.40 ^a	107.63±15.76 ^b
PC	74.44±16.26 ^a	106.66±12.30 ^b

Mean ± Standard deviation.

Different letters in the same column differ significantly ($P<0.05$) by the Tukey's test.

CN: conventional, with 20% fat; CT: control, with 10% fat; PA: with 10% fat and 1.5% of pineapple byproduct; CO: with 10% fat and 5% of canola oil; PC: with 10% fat, 1.5% of pineapple byproduct and 5% of canola oil.

According to the Brazilian Table of Food Composition (TACO), pork back fat (raw) has 73 mg/100g (UNIVERSIDADE ESTADUAL DE CAMPINAS, 2011) and according to the Nutrient Database for Standard Reference of the United States Department of Agriculture (USDA), the cholesterol content of lard (raw) is 95 mg/ 100 g (UNITED STATES, 2011).

Taking into account these two databases and considering 100 g of burger, the reduction of fat from 20 to 10% in raw beef burgers, would result in a cholesterol decrease between 7.3 mg (TACO) and 9.5 mg (USDA table). The results of this study were consistent with the values indicated by the reference tables, since the low-fat burgers (100 g) had a mean cholesterol reduction of 7.62 mg compared to the conventional burger.

Fat reduction showed significant effect in the cholesterol content of cooked products (Table 2). Cholesterol level of CN was significantly higher than those of the low-fat treatments (CT, PA, OC, PC), which showed a mean decrease of 9.15%. The same effect was found by Gök et al. (2011), who evaluated the use of ground poppy seed as a fat replacer on beef burgers and by Muguerza et al. (2001), which reported that products with 20, 25 and 30% of replacing level of pork back fat had significantly lower cholesterol than the control.

5.3.2 Fatty acid profile

The treatment with addition of just pineapple byproduct was not significantly different from the conventional and the low-fat control burgers regarding the fatty acids quantified, except for the palmitoleic acid, wherein PA differed from CT (Table 3). Since the pineapple byproduct used here has a small amount of fat (1.32 g/100 g, chapter 2), its addition was not enough to promote changes in the lipid profile of the low-fat beef burgers.

On the other hand, and as expected, canola oil addition promoted significant alterations in the fatty acid profile of the reformulated products. According to the results, oil addition promoted a decrease in the miristic, palmitic and stearic acids compared to those found in CN and CT. The absence of miristic acid and the lower amounts of palmitic (4.30 g/100 g) and stearic acids (2.09 g/100 g) in the canola oil compared to the amount found in the pork fat (1.30 mg miristic acid, 23.8 palmitic acid, and 13.5 g stearic acid /100 g) (UNITED STATES, 2011), were the responsible for these reductions in CO and PC, indicating that the fatty acid composition of the treatments reflected the characteristic lipid composition of the type of fat/oil used in the formulation. The reduction of the three major saturated fatty acids consequently resulted in CO and PC showing significantly lower SFA contents than the treatments without vegetable oil. Similar results were reported by Choi et al. (2010), who found a significant reduction in the total SFA and in the miristic, palmitic, and stearic acids of reduced-fat frankfurters with rice bran fiber and canola oil (10%) compared to the control. SFAs are known to increase the low density lipoproteins and hence, blood cholesterol level (MATTSON; GRUNDY, 1985). Thus, regarding SFA, canola oil treatments showed nutritional advantages in relation to the other treatments.

Table 3 - Fatty acid composition (g/100 g fatty acids) of raw and cooked burgers with addition of pineapple byproduct and canola oil

	CN	CT	PA	CO	PC
Raw Burger					
Miristic C14:0	1.66±0.08 ^a	1.54±0.20 ^a	1.46±0.20 ^{ab}	1.25±0.29 ^b	1.25±0.24 ^b
Palmitic C16:0	21.92±0.89 ^a	20.46±0.23 ^a	20.95±0.95 ^a	17.61±1.39 ^b	17.54±1.17 ^b
Palmitoleic C16:1	1.37±0.32 ^{ab}	1.29±0.29 ^b	1.42±0.35 ^a	1.06±0.37 ^c	1.10±0.32 ^c
Stearic C18:0	9.08±0.17 ^a	8.93±0.40 ^a	9.01±0.44 ^a	7.52±0.76 ^b	7.40±0.77 ^b
Oleic C18:1	44.82±3.71 ^b	41.54±1.70 ^b	42.51±2.29 ^b	49.55±2.19 ^a	49.00±3.65 ^a
Linoleic C18:2	13.26±1.65 ^{bc}	12.24±1.73 ^c	12.38±1.34 ^c	14.89±0.98 ^a	14.64±0.78 ^{ab}
Linolenic C18:3	0.54±0.11 ^b	0.53±0.14 ^b	0.53±0.15 ^b	1.97±0.25 ^a	1.88±0.27 ^a
ΣSFA	32.66±0.75 ^a	30.93±0.41 ^a	31.43±1.43 ^a	26.38±2.42 ^b	26.19±2.12 ^b
ΣMUFA	46.20±3.49 ^b	42.83±1.55 ^b	43.92±2.38 ^b	50.62±2.10 ^a	50.10±3.56 ^a
ΣPUFA	13.81±1.70 ^b	12.77±1.85 ^b	12.91±1.44 ^b	16.86±0.97 ^a	16.52±0.81 ^a
P/S	0.42±0.06 ^b	0.41±0.06 ^b	0.41±0.06 ^b	0.64±0.08 ^a	0.64±0.08 ^a
n-6/n-3	25.05±5.15 ^a	23.76±4.38 ^a	24.68±6.61 ^a	7.64±1.10 ^b	7.91±1.16 ^b
AI	0.48±0.02 ^a	0.48±0.02 ^a	0.47±0.02 ^a	0.34±0.05 ^b	0.34±0.04 ^b
TI	1.04±0.04 ^a	1.06±0.04 ^a	1.05±0.03 ^a	0.68±0.09 ^b	0.69±0.09 ^b
Cooked Burger					
Miristic C14:0	1.47±0.17 ^a	1.57±0.02 ^a	1.51±0.15 ^a	1.61±0.34 ^a	1.51±0.22 ^a
Palmitic C16:0	20.98±1.39 ^a	20.49±1.71 ^{ab}	20.83±0.46 ^a	17.16±0.88 ^b	17.31±1.43 ^b
Palmitoleic C16:1	1.33±0.30 ^a	1.36±0.37 ^a	1.36±0.31 ^a	1.17±0.34 ^b	1.15±0.34 ^b
Stearic C18:0	9.01±0.57 ^a	8.56±1.52 ^a	8.86±0.37 ^a	8.47±0.93 ^a	8.42±0.62 ^a
Oleic C18:1	43.48±3.72 ^{ab}	39.20±8.99 ^b	41.61±5.31 ^{ab}	47.86±5.36 ^{ab}	51.74±3.29 ^a
Linoleic C18:2	12.41±1.01 ^{bc}	11.70±0.74 ^c	11.66±1.18 ^c	14.11±1.25 ^a	13.52±0.41 ^{ab}
Linolenic C18:3	0.46±0.07 ^b	0.54±0.09 ^b	0.57±0.11 ^b	1.66±0.33 ^a	1.64±0.17 ^a
ΣSFA	31.46±1.78 ^a	30.62±3.20 ^a	31.20±0.49 ^a	27.82±0.57 ^a	27.25±2.07 ^a
ΣMUFA	44.81±3.63 ^{ab}	40.55±8.84 ^b	42.97±5.05 ^{ab}	49.20±5.13 ^{ab}	52.89±3.15 ^a
ΣPUFA	12.87±1.03 ^a	12.25±0.79 ^a	12.23±1.27 ^a	15.77±1.24 ^b	15.16±0.38 ^b
P/S	0.41±0.06 ^b	0.41±0.07 ^b	0.39±0.04 ^b	0.56±0.05 ^a	0.56±0.05 ^a
n-6/n-3	27.34±4.14 ^a	21.93±3.67 ^a	20.66±2.90 ^a	8.76±1.98 ^b	8.28±0.96 ^b
AI	0.46±0.01 ^a	0.51±0.05 ^a	0.49±0.04 ^a	0.38±0.03 ^b	0.35±0.04 ^b
TI	1.05±0.02 ^a	1.11±0.04 ^a	1.08±0.06 ^a	0.78±0.05 ^b	0.72±0.08 ^b

Mean ± Standard deviation.

Different letters in the same row differ significantly ($P<0.05$) by the Tukey's test.

AI: atherogenic index; TI: thrombogenic index.

CN: conventional, with 20% fat; CT: control, with 10% fat; PA: with 10% fat and 1.5% of pineapple byproduct; CO: with 10% fat and 5% of canola oil; PC: with 10% fat, 1.5% of pineapple byproduct and 5% of canola oil.

In cooked burgers, among the saturated fatty acids, just palmitic acid was significantly lower in the canola oil treatments compared to CN and PA, and consequently, no significant difference was observed in the SFA content among treatments. The different mechanisms that occur during heat treatment, such as water and fat loss, hydrolysis, oxidation, and polymerization of the triacylglycerol molecule (TAKEOKA; FULL; DAO, 1997), may have promoted some alterations in the lipid profile of the products, leading to different behaviors regarding the SFA content of raw and cooked burgers. Alterations in the lipid composition of

raw and cooked beef burgers were also observed in the study of Scheeder et al. (2001), using electric grill at 200 °C, for 8 min, to reach the final temperature of 77 °C, which is a cooking procedure similar to that used in the present study.

In raw burgers of CO and PC, MUFA levels increased compared to the values found in treatments without canola oil incorporation. Despite the significantly lower content of palmitoleic acid in these two treatments, the expressive content of oleic acid in canola oil (61.74 g/100 g) (UNITED STATES, 2011) was certainly the factor that caused this increment in the amount of MUFAs. MUFAs are hypocholesterolemic and among the unsaturated fatty acids, they display more beneficial effects than PUFA regarding the increase in the HDL-cholesterol, which is an important factor for the prevention of cardiovascular diseases (MATTSON; GRUNDY, 1985). Increase in monounsaturated fatty acids was also reported by Pelser et al. (2007), evaluating the use of canola oil in low-fat Dutch style fermented sausages.

In cooked burger, a slight increase in the content of MUFA was also observed, however it was not significant. Differences in the behavior of the MUFA content between raw and cooked burgers, as previously reported, were probably a result of the different events that occurs during cooking, which affected the fatty acid composition of the cooked burgers. In this case, an important factor that may have caused the reduction of MUFA in cooked products is the initiation of the lipid peroxidation of unsaturated fatty acids during the heat treatment (ELIF BILEK; TURHAN, 2009).

The partial substitution of animal fat by canola oil resulted in higher PUFA contents in raw and cooked burgers. Both linoleic and linolenic acids increased in treatments with vegetable oil addition, and according to the results, this PUFA increase was mainly caused by the linolenic acid, since its amount was raised approximately 3.61 and 3.15 times in raw burgers and cooked burgers, respectively, compared to the other treatments. As MUFAs, PUFAs are also known to reduce plasma cholesterol, acting positively in the prevention of cardiovascular diseases (MATTSON; GRUNDY, 1985). These results are similar to the findings of Asuming-Bediako et al. (2014), who reported higher levels of C18:2 and C18:3 in sausages with rapeseed oil compared to the formulation with back fat.

Unlike the results observed for SFA and MUFA, there was no change in the PUFA behavior of raw and cooked burgers, since in both ways PUFA was significantly higher in OC and PC. According to Ono, Berry, and Paroczay (1985), this may be explained by the fact that more saturated than unsaturated fatty acids are lost during heating, because the latter have a

greater association with structural components and thus are not readily released during the cooking process.

Besides the evaluation of the fatty acids, the nutritional quality of the lipid profile can be evaluated using different indexes, such as the PUFA/SFA, and n-6/n-3 ratios, and the atherogenic, and thrombogenic indexes (Table 3). Considering the improvement in the fatty acid profile of raw and cooked burger with canola oil incorporation, these treatments showed better ratios than the results found in the other formulations. It is recommended that PUFA/SFA ratio should be higher than 0.45, since lower ratios in the diet may increase the incidence of cardiovascular disease (CIFUNI et al., 2004). PC and CO showed a significant increase in the PUFA/SFA compared to the other treatments, resulting in higher ratios (0.56 to 0.64) than the recommended value, while treatments without vegetable oil incorporation showed levels below 0.42. In agreement with the results present here, the use of flaxseed oil in beef patties (ELIF BILEK; TURHAN, 2009), rapeseed oil in UK-style sausages (ASUMING-BEDIAKO et al., 2014), and olive, corn, canola and soybean oil in frankfurters (CHOI et al., 2010) increased the PUFA/SFA ratios to values higher than 0.45.

According to Simopoulos (2002), high n-6/n-3 ratios are associated with the occurrence of some health problems, including cardiovascular, inflammatory and autoimmune diseases, whereas lower ratios exert suppressive effects. For prevention of cardiovascular disease, the recommendation is to reduce the value to less than 4 (SALCEDO-SANDOVAL et al., 2014). Considering that some meats naturally have the n-6/n-3 ratio higher than this value (WOOD et al., 2004), the addition of 5% of canola oil emulsion was not enough to reduce the characteristic high ratio of meats to the recommended level, but its incorporation in raw and cooked beef burgers significantly reduced the ratios from 20.66-27.34 in the treatments CN, CT and PA, to values between 7.64-8.76.

Atherogenic (AI) and thrombogenic indexes (TI) are also used to measure the propensity of a diet or food to influence the incidence of coronary heart disease and the differences of these indexes for the PUFA/SFA and n-6/n-3 ratios are based on the fact that they consider the different promoting and suppressive effects of the fatty acids regarding these two processes. The SFAs that are thrombogenic are not quite the same as those that are atherogenic and unsaturated fatty acids present different degrees of protection against atheroma and thrombosis (ULBRICHT; SOUTHGATE, 1991). Similarly to the other ratios, the use of canola oil in the two formulations of beef burger (CO and PC) significantly decreased the AI and TI of raw and cooked burgers compared to the other treatments. Rodríguez-Carpena et al. (2012) reported AI values of 0.41 for control pork patty (with pork

back fat) and 0.25, 0.23 and 0.24 for patties with avocado, sunflower and olive oils, which are in agreement with the results presented here. Reduction in TI was observed in the study of López-López et al. (2009), who found values of 0.85 for control and 0.57 for frankfurters with olive oil.

5.2.3 Lipid oxidation

The oxidative stability of the samples was evaluated by measuring the malonaldehyde, which was determined and quantified by its reaction with the thiobarbituric acid. MDA is one of the major secondary products of the lipid oxidation process, being formed by hydroperoxides decomposition (BONNES-TAOUREL; GUÉRIN; TORREILLES, 1992).

According to the results, there were significant effects of the formulation, storage time, and interaction between both factors in raw burgers. Overall, there was no effect of the formulations on the malonaldehyde content of raw burgers after one day of frozen storage (Figure 1). This result probably occurred because the development of lipid oxidation after just one day of the burger manufacture was not enough to show the possible effects of the fat reduction and partial fat substitution by pineapple byproduct and/or canola oil on the oxidative stability of the products.

Figure 1 - MDA content (mg/kg) of raw (A) and cooked (B) burgers with addition of pineapple byproduct and canola oil

CN: conventional, with 20% fat; CT: control, with 10% fat; PA: with 10% fat and 1.5% of pineapple byproduct; CO: with 10% fat and 5% of canola oil; PC: with 10% fat, 1.5% of pineapple byproduct and 5% of canola oil.

Even after 30 days of storage, treatments were not significantly different. This result could be due to the use of frozen storage, to the fact that the samples were raw and to the short storage time that do not favor the lipid oxidation, resulting in treatments with similar malonaldehyde contents.

From 60 days until the end of the storage time, treatments showed significant difference. After 60 days, CO was the most oxidized sample, while after 90 and 120 days PC-burgers showed the highest MDA values. In the last three evaluation times (60, 90 and 120 days), the same treatments (CN, CO and PC) were the most susceptible to the occurrence of lipid oxidation. PA and CT were the two treatments that had the lowest amounts of MDA throughout the storage time, which, after 120 days, showed values of 0.65 and 0.88 mg MDA/kg sample, respectively.

For cooked burgers, there was significant effect of the treatments, storage time, and interaction between them. As observed for raw products, cooked burgers, even after heat treatment, did not show significant difference among treatments one day after the burger manufacture (Figure 1). In general, cooked treatments showed the same trend observed in raw samples. With 30 days of storage, CN and PC showed higher MDA content than the other treatments, and CT had the lowest value. After 60 and 90 days, full-fat burgers and the two formulations with canola oil were more oxidized than the low-fat control and the treatment with pineapple byproduct. Finally, at the end of the storage time, the treatment with addition of canola oil in combination with pineapple byproduct presented significantly higher MDA content than all the other treatments. CN and CO showed intermediate oxidation values, followed by CT and PA, which had the lowest MDA content.

These results are indicative that the addition of canola oil, with or without pineapple byproduct, negatively affected the oxidative stability of raw and cooked burgers. This behavior can be attributed to the increased susceptibility of unsaturated fatty acids to lipid oxidation (double bonds are reactive sites in the molecule), present in high quantities when animal fat was partially replaced by the vegetable oil. Similarly to the present study, Choi et al. (2010) reported higher TBARS values of reduced-fat frankfurters with addition of canola oil than those of the control, containing no added vegetable oil.

Despite the enhance of the lipid oxidation, CO and PC showed, at the end of the storage time (120 days), MDA values of 1.19 and 1.41 mg/kg of raw burger, respectively, and 1.33 and 1.50 mg/kg of cooked burger, respectively. These values are below the MDA threshold for acceptability, since according to Trindade, Mancini-Filho, and Villavicencio

(2009), 2 mg MDA/ kg is believed to be the threshold that can indicate loss of sensory quality and perception of oxidation by consumers.

Regarding the high MDA values found in the burgers of the conventional treatment, although animal fat is high in saturated fat, which is more stable to oxidation, pork back fat has 56.3% of unsaturated lipids (45.1% MUFA and 11.2% PUFA) (UNITED STATES, 2011). As CN has twice the amount of fat of the other treatments, it was expected that the lipid oxidation of these burgers would occur at higher speeds, resulting in higher MDA content.

The lipid oxidation of low-fat control and the treatment with pineapple byproduct was retarded in raw and cooked burgers compared to the other treatments due to the reduced amount of animal fat in both formulations and, in the case of PA, probably because pineapple byproduct has phenolic compounds (3.78 mg gallic acid equivalent/g pineapple byproduct, chapter 2), with antioxidant activity (DPPH: 5.76 μmol Trolox/g pineapple byproduct; ABTS: 13.46 μmol Trolox/g pineapple byproduct, chapter 2) that could have helped to protect the burgers against the lipid oxidation.

MDA content of beef burgers of all treatments increased throughout the storage time, with averages at the beginning of the experiment of 0.05 and 0.08 mg MDA/kg of raw and cooked burger, respectively, and averages at the end of the frozen storage of 1.02 and 1.18 mg MDA/kg of raw and cooked meat, respectively. The development of oxidative rancidity occurs even during the frozen storage because while the rate of the deteriorative reactions (microbiological and enzymatic) can be inhibited by low temperatures, lipid oxidation are slowed, but not completely prevented (GRAU et al., 2000). López-López et al. (2010), studying low-fat beef burger with addition of olive oil and wakame (seaweed), also observed increase in MDA values of beef burger during frozen storage, which started with values ranging from 0.43 to 0.53 mg MDA/kg raw burger at the beginning of the experiment (4 days) and showed values between 0.73 to 0.87 mg MDA/kg raw burger at the end of the storage time (152 days). The initial oxidation values found by these authors are much higher than those present here. The causes for these differences can be related to the time of storage, type of vegetable oil used, the burger formulation, the oxidative condition of the raw material (beef, fat, and oil), but in this case, it could be also due to the method used to evaluate the lipid oxidation, since López-López et al. (2010) used the traditional TBARS method (colorimetric), which is known for its lack of specificity, leading to overestimated results, while the present study used the MDA quantification by HPLC that is a more sensitive and specific method.

5.4 Conclusions

The use of canola oil in combination with pineapple byproduct as partial fat replacers led to the development of healthier cooked burgers as a result of a cholesterol reduction and an improvement in the nutritional quality of the lipid profile, observed through an increase in the PUFA/SFA ratio and a decrease in the n-6/n-3 ratio and in the atherogenic and thrombogenic indexes.

Despite the highest MDA values found in CO and PC, canola oil incorporation did not reduce the shelf life of beef burgers in terms of lipid oxidation, since after 120 days of storage, their values were lower than the MDA threshold for consumer's acceptance.

Thus, the results of this study highlight the application of canola oil and pineapple byproduct as food ingredients in the development of healthier beef burgers.

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6 CHARACTERISATION AND POTENTIAL APPLICATION OF PINEAPPLE POMACE IN AN EXTRUDED PRODUCT FOR FIBRE ENHANCEMENT

Chapter published in Food Chemistry.

SELANI, M.M.; CANNIATTI-BRAZACA, S.G.; DIAS, C.T.S.; RATNAYAKE, W.S.; FLORES, R.A.; BIANCHINI, A. Characterisation and potential application of pineapple pomace in an extruded product for fibre enhancement. **Food Chemistry**, London, v. 163, p. 23-30, 2014.

Abstract

This study characterised pineapple pomace (PP) and evaluated its application in extrusion to enhance fibre content of the final product. The pomace had low fat (0.61%) and high dietary fibre (45.22%), showing its potential for fibre enrichment of nutritionally poor products, as some extruded snacks. Results also showed low microbiological counts, water activity, and pH indicating good microbiological quality and low risk of physicochemical deterioration. During extrusion, pomace (0%, 10.5% and 21%), moisture (14%, 15% and 16%) and temperature (140 and 160 °C) were evaluated. The PP addition decreased expansion and luminosity; while increasing redness of the extrudates compared to the control (0% pomace/14% moisture/140 °C). When hardness, yellowness, water absorption, and bulk density were compared to the control, there was no effect ($p > 0.05$) of 10.5% PP addition on the extrudates, indicating that, at this level, PP could be added without affecting the properties of the final extruded product.

Keywords: Pineapple pomace; Dietary fibre; Food ingredient; Extruded product

6.1 Introduction

Pineapple world production reached 21.8 million tons in 2011 (FAO, 2011), and most of its production is used for processing as fruits salads, juices, concentrates, and jams. During processing, large amounts of byproducts, consisting mainly of peel and pomace are generated, representing about 25–35% of the fruit weight (LARRAURI; RUPEREZ; CALIXTO, 1997). Since most of these byproducts have no specific destination, they may be inappropriately disposed causing environmental issues. Consequently, it is of vital importance to reuse industrial byproducts in order to improve the process economics and its sustainability.

It is well-known that dietary fibre plays an important role in human health, promoting several physiological and metabolic positive effects (RANINEN et al., 2011). The insoluble dietary fibre acts as a bulking agent, normalizing intestinal motility, preventing constipation, while soluble fibre is associated to decreasing the intestinal absorption of cholesterol and glucose (RODRÍGUEZ; MEGIAS; BAENA, 2003). Due to all of these benefits of dietary fibre intake, a tendency in the development of products enriched with fibre or with specific fibre claims has already been observed for some time. According to the Food and Drug Administration (FDA) (2013), to have a product with a “high source of fibre” and “good

source of fibre'' claim, it must contain, respectively, 20% or more fibre and 10-19% of fibre of the recommended daily value for dietary fibre in a serving size.

About 76% of pineapple byproduct (peel and heart) is fibre, from which 99.2% is the insoluble fraction and 0.8% is the soluble fraction (MARTÍNEZ et al., 2012). As pineapple pomace contains valuable sources of dietary fibre, it could be used as a potential food ingredient to improve nutritional quality of foods. Furthermore, fibres have technological properties, such as water holding capacity (WHC), swelling capacity (SWC) and oil holding capacity, which can be useful in products that require hydration, to avoid syneresis, improve yield, stabilize high fat food products and emulsions, and also to modify texture and viscosity (ELLEUCH et al., 2011). One alternative would be to use these byproducts as ingredients in selected food processes, i.e. extrusion processing.

Extrusion is an attractive choice because of its versatility (wide range of food products applications), high productivity, relative low cost, energy efficiency and lack of effluents (ALTAN; MCCARTHY; MASKAN, 2008). Furthermore, extrusion-cooked products tend to be nutritionally poor since they are energy dense, and low in health promoting ingredients. The extrusion process itself promotes the gelatinization and depolymerisation of the starch, leading to an increase in the amount of easily digestible carbohydrates and resulting in a product with high glycemic index (BRENNAN et al., 2013a).

Due to the characteristics of these products, some studies have evaluated the addition of food processing byproducts in extruded foods as sources of fibre and bioactive compounds. The byproducts include orange peel, grape seed and tomato pomace (YAĞCI; GÖĞÜŞ, 2008); a mixture of cauliflower florets, curd, stem and leaves (STOJCESKA et al., 2008); and carrot pomace (KUMAR; SARKAR; SHARMA, 2010). The addition of these byproducts promoted some alterations in the characteristics of the extrudates, but in all of the studies, good sensory acceptance was observed. Brennan et al. (2013b) used β -glucan rich materials from mushroom and barley processing as ingredients in extruded snacks and reported that the inclusion of this fibre promoted a reduction in the glycemic response.

Since pineapple processing produces significant amounts of fibre rich pomace and currently there is lack of studies about its application in extruded products, the use of this byproduct in extrusion would be an interesting option for fibre enrichment. Besides, it would benefit the pineapple processing operations by making them more economically sustainable. Thus, the objective of this study was to characterise and evaluate the performance of pineapple pomace as an ingredient in extruded products.

6.2 Material and Methods

6.2.1 Production of pineapple pomace

Three different batches of mature pineapple (*Annanas comosus*), variety Gold (Del Monte Gold, Costa Rica), were purchased from local stores in Lincoln, NE, USA, during September 2012. To produce the byproduct, referred herein simply as pomace, fruits were processed at The Food Processing Center Pilot Plants of the University of Nebraska-Lincoln. First, the fruits were sanitized with 200 ppm of sodium hypochlorite, rinsed with water, and cut by hand into small pieces with peel. The pieces were introduced in a juice extractor (Speed Troll, Sterling Electric Inc., Irvine, CA, USA), where the juice was expelled and the pomace collected. The pineapple pomace (PP) (peel and pomace) was freeze-dried (Thermovac, Long Island, NY, USA) for 72 h under vacuum condition, ground using a knife mill (Mini Mill, Thomas Wiley, Swedesboro, NJ, USA), passed through a 40-mesh stainless steel sieve (420 μm), and stored into sealed plastic containers at $-20\text{ }^{\circ}\text{C}$ for further analyses. The particle size of these samples (Table 1) was determined in triplicate by laser diffraction in a Malvern Mastersizer 3000 (Malvern Instruments, Worcestershire, UK), using the dry dispersion method (Aero S dispersion cell), with a refractive index of 1.53, an air pressure of 4 bar and a sample feed of 50%.

Table 1 - Particle size distribution of freeze-dried pineapple pomace

Size (μm)	% Undersize particles*
211	48.94 $\pm$ 2.13
454	87.13 $\pm$ 0.30
666	97.06 $\pm$ 0.38
859	99.05 $\pm$ 0.43
1110	99.51 $\pm$ 0.36
3080	100.00 $\pm$ 0.00

* Percentage of particles with diameter under the size (μm) described in the first column of this table.
Mean $\pm$ Standard deviation.

6.2.2 Characterization of pineapple pomace

6.2.2.1 Chemical analyses

Ash was determined according to the Association of Official Analytical Chemists – AOAC, method 940.26 (AOAC, 2000), using a Thermolyne 30400 Furnace (Thermo Scientific, Waltham, MA, USA). Moisture was determined using a vacuum Thermolyne Oven Series 9000 (Thermo Scientific, Waltham, MA, USA) following the method 924.06 (AOAC, 2000). Soluble (SDF) and insoluble dietary fibre (IDF) were determined according to the method 960.52 (AOAC, 2000). Protein was calculated from the nitrogen content by the

Dumas combustion method (Leco FP-528, Leco Corporation, St. Joseph, MI, USA) using a conversion factor of 6.25 and fat was determined by the Soxtec method (Soxtec 2043, Foss, Hillerød, Denmark), both according to the manufacturer's instructions. Carbohydrate content was calculated by difference. The analyses were carried out in triplicate.

6.2.2.2 Physicochemical analyses

The pH of the samples was measured in a suspension obtained from a blend of 10 g of sample with 100 mL of deionised water, using a pH metre (Orion 2 Star, Thermo Electron Corporation, Beverly, MA, USA). Acidity was determined by titration with 0.1 N sodium hydroxide and the results were reported as g of citric acid/100 g of sample. Water activity (A_w) was determined using an Aqualab instrument (Aqualab Series 3TE, Decagon devices Inc., Pullman, WA, USA) at 25 °C. Colour was measured with a colorimeter (Minolta CR-300, Konica Minolta, Osaka, Japan), with D65 as illuminant, based on the CIELAB L^* , a^* , b^* colour space. The instrument was calibrated using a colour standard (white), with $Y = 92.7$, $x = 0.3162$ and $y = 0.3325$, provided by the manufacturer. Colour readings were taken in different points on the surface of the byproduct powder. The analyses were carried out in triplicate.

6.2.2.3 Functional properties

Water holding capacity (WHC) and oil holding capacity (OHC) were determined in triplicate by the method described by Carcea-Bencini (1986), with the following modifications. One gram of pomace sample was stirred in 10 mL of distilled water (for WHC) or canola oil (Pure Wesson, ConAgra Foods, Omaha, NE, USA) (for OHC), centrifuged (Sorvall Legend XTR Thermo Scientific, Waltham, MA, USA) at 2200g for 30 min and the supernatant was carefully eliminated. Water holding capacity was reported as the number of grams of water held by 1 g of sample and oil holding capacity was reported as the number of grams of oil held by 1 g of sample.

6.2.2.4 Microbiological analyses

Microbial counts were determined by diluting 25 g of sample in 225 mL of phosphate sterile buffered peptone water (BPW) (Acumedia, Lansing, MI, USA) to achieve the 1:10 dilution. Following dilution, samples were blended using a Stomacher (Seward Laboratory Systems, Bohemia, NY, USA), for 1 min. Further serial dilutions were prepared in BPW for

all of the microbial determinations. Total plate counts (TPC) were determined by plating the diluted samples on APC Petrifilm plates (3 M, St. Paul, MN, USA) followed by incubation at 35 °C for 48 h. *Escherichia coli* and thermotolerant coliforms were counted by plating samples on *E. coli*/coliform petrifilm plates (3 M, St. Paul, MN, USA) following incubation at 45 °C for 24 h. Molds and yeasts counts were determined after plating samples on Dichloran Rose Bengal Chloramphenicol agar (DRBC) (Acumedia, Lansing, MI, USA) and incubating at 25 °C for 5 days. All these determinations were carried out in duplicate for each dilution. *Salmonella* testing was performed by a pre-enrichment with BPW at 37 °C for 24 h, followed by a selective enrichment step with Tetrathionate Broth (TTB) (Acumedia, Lansing, MI, USA) at 41 °C for 24 h. Enriched samples were then applied to a pathogen-specific test strips (RapidCheck, SDIX Newark, DE, USA), according to the manufacturer's directions.

6.2.3 Extrusion experiments

6.2.3.1 Blend preparation

Corn flour (Bunge Milling, St. Louis, MO, USA) was replaced by pineapple pomace (dry basis) at levels of 21% and 10.5%. These levels were chosen with the aim to deliver 10% (claim of “good source of fibre”) (FDA, 2013) and 5% of the recommended daily value for dietary fibre (25 g/day) in a serving size (28.35 g). The treatments were adjusted to moisture levels of 14, 15 and 16% (w/w) in a mixer (Hobart, Troy, OH, USA) for 5 min. After mixing, the samples were placed in polypropylene plastic bags (Uline Poly Bags, S-1255) and stored at 4 °C overnight to ensure homogeneous moisture distribution.

6.2.3.2 Extrusion

Three extrusion trials were done using a single screw laboratory extruder (C.W. Brabender, model 2003 GR-8, South Hackensack, NJ, USA) with a die opening of 3 mm and a screw speed of 220 rpm.

The extruder screw had a compression ratio of 3:1, a length/diameter ratio of 20/1, length of 38 cm, and a diameter of 19 mm. The temperature of the first (feed) zone was set to 80 °C, while the second (metering) was set to 90 °C for all trials. Two temperatures at the third (compression) zone were evaluated: 140 and 160 °C. When the extrusion response parameters were stable, extrudates were collected for about 3 min. The samples were dried for 10 min at 100 °C in a drier (Wenger, Sabetha, KS, USA) to standardise the moisture for texture analysis. Then, the material was stored in polypropylene plastic bags (Uline Poly Bags, S-1255) at room temperature (21 °C) for further analysis.

During extrusion, parameters including torque, temperature and pressure were recorded. Extrusion rate was determined by collecting the extrudates for 1 min, and weighing the material to determine the mass extruded per minute. Specific mechanical energy (SME), the mechanical energy input per unit mass of the extrudate, was calculated by dividing the net power input to the screw by the extrudate flow rate according to Eq. (1). Specific mechanical energy (KIRBY et al., 1988).

$$\text{SME (Wh/kg)} = \frac{\text{screw speed (radians/s)} \cdot \text{torque (N m)}}{\text{Mass flow rate (kg/h)}} \quad (1)$$

6.2.3.3 Expansion ratio

The diameter of 20 pieces of extruded products was measured using a caliper (Mitutoyo, Tokyo, Japan) and the expansion ratio was calculated by dividing the average diameter of the products by the diameter of the die.

6.2.3.4 Bulk density

Bulk density (BD) (g/cm^3) of 10 pieces of extrudates was calculated according to Eq. (2) (Bulk density (g/cm^3) (ALVAREZ-MARTINEZ; KONDURY; KAPER, 1988), where m is mass (g) of a length L (cm) of extrudate, with diameter d (cm).

$$\text{BD} = \frac{4m}{\pi d^2 L} \quad (2)$$

6.2.3.5 Water absorption and solubility indices

Water absorption index (WAI) and water solubility index (WSI) were determined in triplicate, according to the method of Anderson et al. (1969). The WSI was the weight of dry solids in the supernatant reported as percentage of the original weight of sample. The WAI was the weight of gel obtained after removal of the supernatant per unit weight of original dry solids.

6.2.3.6 Colour

Colour was measured using the method described in Section 6.2.2.2, using the CIELAB L^* , a^* , b^* colour space. For this analysis, the extruded product was ground (Mini

Mill, Thomas Wiley, Swedesboro, NJ, USA) and passed through a 40-mesh sieve. Colour readings were taken from 3 separate points on the surface of the powder.

6.2.3.7 Moisture content

The moisture content of the extrudates was determined in triplicate, according to the American Association of Cereal Chemists – AACC, method 44–15A (AACC, 2000).

6.2.3.8 Texture analysis

The hardness of the extruded samples was determined by a three-point bend test, using a texture analyser (TMS-PRO, Food Technology Corporation, Sterling, VA, USA), equipped with a TMS lightweight three-point bend probe. Hardness (N) was determined by measuring the maximum force required to break the extruded samples (50 mm long). The probe was set to move at a speed of 0.8 mm/s for a distance of 10 mm and the distance between the two supports was 20 mm. Six measurements were performed for each treatment.

6.2.4 Experimental design and statistical analysis

The study was a randomized block design, with three blocks (three independent extrusion processes). The statistical analysis was performed according to the set of 14 treatments. The data was subjected to exploratory analysis, to residue analysis, to test of variance homogeneity, to test for outliers and to range of the measured variable. For the texture analysis, a logarithmic transformation (base 10) was performed. The comparison among the means was done with transformed data and the results presented in the original scale. Once the fit of the data was assessed, significant differences among samples were determined by analysis of variance (ANOVA) and Tukey's test ($P < 0.05$), using the software SAS.

6.3 Results and discussion

6.3.1 Characterisation of pineapple pomace

6.3.1.1 Chemical analyses

Moisture and protein content of the freeze-dried pineapple pomace were $3.77 \pm 0.52\%$ and $4.71 \pm 0.28\%$ (Table 2), respectively. The protein value obtained in this study was similar to the protein content of the pineapple byproduct (peel and heart) studied by Martínez et al. (2012) (4%). The pomace had low fat content ($0.61 \pm 0.14\%$) (Table 2), which is in agreement with the 0.69% reported by Sousa et al., (2011) for pineapple residue obtained

from a fruit pulp industry. The present study found higher values of ash ($2.24 \pm 0.58\%$) (Table 2) when compared to industrial pineapple residue (0.53%) (SOUSA et al., 2011) and lower values compared to pineapple fibre concentrate (4.5%) (MARTÍNEZ et al., 2012). Differences in fruit variety can influence the content of ash, but, according to Lombardi-Boccia et al. (2004), the main factor influencing mineral concentration in vegetable products is the type of soil used for cultivation. According to the proximate composition, the major compound of the pineapple pomace was fibre ($45.22 \pm 3.62\%$), where 98.28% of it was the insoluble fraction (Table 2). This result is in agreement with those reported by Huang, Chow, and Fang (2011), who found 42.2% of total dietary fibre in pineapple peels, with the insoluble fraction being its main constituent (86.02%). However, Larrauri, Rupérez and Calixto (1997), studying pineapple shell, found 70.61% of total dietary fibre with 99.28% of it being insoluble fibre. Differences in fibre content can also be attributed to differences in cultivars and growing conditions (FIGUEROLA et al., 2005). The carbohydrate content in this study was obtained by difference and it is similar to that reported by Huang, Chow, and Fang (2011), who found 42.3% in pineapple peel.

Table 2 - Chemical composition of freeze-dried pineapple pomace

Component	Percentage (% d.b.)
Moisture	3.77 ± 0.52
Protein	4.71 ± 0.28
Fat	0.61 ± 0.14
Ash	2.24 ± 0.58
Total dietary fiber	45.22 ± 3.62
Insoluble dietary fiber	44.44 ± 3.60
Soluble dietary fiber	0.78 ± 0.10
Carbohydrates*	43.46

Mean $\pm$ Standard deviation.

*By difference.

d.b.: dry basis.

Thus, according to the proximate composition, pineapple pomace can be a promising ingredient for fibre enrichment, leading to the development of healthier products.

6.3.1.2 Physicochemical analyses

The pineapple pomace had a pH of 3.86 ± 0.07 (Table 3), which is similar to the pH of Gold pineapple (3.77) (RAMSAROOP; SAULO, 2007), variety used in this study to obtain the pomace. The water activity of the samples averaged 0.14 ± 0.06 (Table 3) which is lower than those reported by Prakongpan, Nitithamyong and Luangpituksa (2002) who found 0.24

for pineapple core dietary fibre and 0.28 for pineapple core cellulose. Considering the low pH and water activity of the material evaluated in this study, it has low risk of food deterioration by microorganisms, enzymes or nonezymatic reactions.

Table 3 – Physicochemical composition and functional properties of freeze-dried pineapple pomace

Physicochemical parameters	Pineapple pomace
pH	3.86 ± 0.07
Titrateable acidity (g citric acid/100 g)	2.01 ± 0.23
Aw	0.14 ± 0.06
Color	
L*	75.63 ± 3.07
a*	-0.10 ± 1.79
b*	26.91 ± 2.35
Functional properties	
WHC (g water/g sample)	5.32 ± 0.67
OHC (g oil/g sample)	2.10 ± 0.17

Mean $\pm$ Standard deviation.

WHC: water holding capacity; OHC: oil holding capacity.

Titrateable acidity was quantified at 2.01 ± 0.23 g citric acid/100 g sample (Table 3). Costa et al. (2007) found 2.53% and 2.98% of citric acid in pineapple peel and pineapple pomace, respectively, values slightly higher than reported here. As most fruits mature, the acidity decreases, and the sugar content increases. Therefore, differences between acidity values may have been caused by variations in fruit ripeness and/or differences in pineapple varieties.

Processed and dried pomace was light in colour, i.e., high L^* value (75.63 ± 3.07) (Table 3), which is a desirable attribute because ingredients with dark colours could limit potential food applications. Regarding the a^* and b^* colour parameters, the sample presented values of -0.1 ± 1.79 and 26.91 ± 2.35 , respectively.

6.3.1.3 Functional properties

The pineapple pomace showed a WHC value of 5.32 ± 0.67 g water/g sample (Table 3), which is lower than the values reported by Prakongpan, Nitithamyong and Luangpituksa (2002), using a similar method for WHC, for pineapple dietary fibre (10.30–12.16 g water/g sample). Differences between reported values more likely were due to differences in the material used for such analysis, since the study of Prakongpan, Nitithamyong and Luangpituksa (2002) evaluated dietary fibre extracted from pineapple pomace, as opposed to the pomace itself. Moreover, the hydration properties could be affected

by the chemical structure of the polysaccharides present in the material, and other factors such as porosity, particle size, ionic form, pH, temperature, ionic strength and type of ions in solution (ELLEUCH et al., 2011). When comparing the WHC value of pineapple pomace reported here with other fruit byproducts, it is higher than lemon peel (1.74–1.85 g water/g sample), orange peel (1.65 g water/g sample) and apple pomace (1.62–1.87 g water/g sample) (FIGUEROLA et al., 2005); and lower than peach pomace (9.2–12.1 g water/g sample) (GRIGELMO-MIGUEL; GORINSTEIN; MARTÍN-BELLOSO, 1999). Besides the plant species, these differences could be due to differences among processing methods. Therefore, because the pineapple pomace as described here has the capacity of holding 5.32 g of its own weight in water, it suggests that it can be used as a functional ingredient in products that require hydration, to avoid syneresis, improve yield, and modify texture and viscosity (ELLEUCH et al., 2011).

The pineapple pomace showed an OHC of 2.01 ± 0.23 g oil/g sample (Table 3). Huang, Chow, and Fang (2011) reported the OHC of the insoluble dietary fibre fraction of pineapple peel to be 5.84 g oil/g sample. This characteristic is a function of the surface properties, overall charge density, thickness, hydrophobic nature of the fibre particle (FIGUEROLA et al., 2005). When compared to other fruits, the OHC results found here for pineapple pomace are higher than the values of peach pomace (1.02–1.11 g oil/g sample) (GRIGELMO-MIGUEL; GORINSTEIN; MARTÍN-BELLOSO, 1999) and lower than pomegranate bagasse (5.9 g oil/g sample) (VIUDA-MARTOS et al., 2012) and lemon byproduct (6.58–6.81 g oil/g sample) (LARIO et al., 2004). According to Kuntz (1994), ingredients with a high OHC allow the stabilization of high fat food products and emulsions. Due to the low OHC values, pineapple pomace does not present potential to be used as an ingredient for these purposes.

6.3.1.4 Microbiological analyses

According to Brackett and Splittstoesser (2001), frozen fruits and vegetables have aerobic plate counts (APC) below 5×10^4 CFU/g. The result found in this study for APC (5.6×10^3 CFU/g) is within the range indicated by these authors. Brackett and Splittstoesser (2001) also reported that coliforms and enterococci are part of the normal flora of plant products, and populations of 10^2 or 10^3 CFU/g of processed products are not uncommon. The present study showed counts of <10 CFU/g for thermotolerant coliform and *E. coli*. Yeast and mold counts in the pineapple pomace were 1.1×10^5 CFU/g and 2.9×10^4 CFU/g, respectively. Low pH and acidity of many fruits are the main factors that influence the

composition of their microflora, and, as most yeasts and molds grow well under acid conditions, fungi are often the predominant microorganisms in fruit products. On the other hand, the acidity of fruits is an obstacle for the growth of the more common foodborne pathogens, such as *Salmonella* and *Shigella*, which do not survive in low-pH environment (BRACKETT; SPLITTSTOESSER 2001). The absence of *Salmonella* spp. in PP is in agreement with that.

6.3.2 Extrusion experiments

The treatments where fibre was added along with 14% of moisture led the extruder to unstable flow and blockage, which made it impossible to obtain samples for those conditions. This may have happened because the pineapple pomace has high amount of fibre, which has as one of its functional properties the capacity to absorb water, preventing the corn starch from being hydrated. This could have resulted in a low level of gelatinized starch, due to insufficient water for gelatinization (LUE; HSIEH; HUFF, 1991). According to Mościcki and Wójtowicz (2011), in extrusion-cooking, mixtures with low water content and high viscosity can overheat and adhere to the cylinder walls or screw, leading to extruder jamming. Thereby, in this study only the results for 14% of moisture and no fibre will be presented and considered as the control for the extrusion, since at this moisture content, extrudates were produced with better quality (expansion, air bubbles formation and visual appearance), when compared to those extruded at 15% and 16% of moisture and no fibre.

6.3.2.1 Process conditions

All the process conditions used during extrusion, all parameters measured during extrusion runs, the extrusion rates and the specific mechanical energy (SME) calculated are presented in Table 4.

In general, the values of pressure, torque, extrusion rate and SME decreased with the increase of temperature. Viscosity is temperature-dependent, since as the mixture gets cooler viscosity increases (KARKLE et al., 2012). Therefore, an increase in temperature causes a reduction in product viscosity, requiring less torque, which leads to reduced SME and less pressure (CHANG et al., 1999). When the pineapple pomace was added to the product, all those parameters also decreased with the level of addition. This could have happened due to the presence of sugar in the pomace, since according to Karkle et al. (2012), low molecular weight carbohydrates can reduce the melt viscosity, acting as plasticizers. The increase in moisture content caused a decrease in torque and SME for all treatments with no fibre added,

which was expected because water also acts as a plasticizer, reducing melt viscosity and promoting lower starch gelatinization (ILO et al., 1996). However, for the treatments with added fibre, in general these two parameters increased with an increase in feed moisture content. Fibres and sugars have the capacity of absorb water, reducing its use for corn starch hydration, which probably promoted higher viscosity, consequently requiring more mechanical energy input to the process.

Table 4 - Extrusion parameters of the extruded products

PP (%)	Moisture (%)	Temperature (°C)*	Torque (Nm)	Pressure (psi)	Extrusion rate (g/min)	SME (W h/kg)
0	14	140	41.43	1.397.87	73.77	214.89
	15	140	39.93	1.346.40	75.58	201.79
	16	140	37.00	1.203.73	75.58	188.06
	14	160	37.90	1.046.83	75.39	192.99
	15	160	34.10	959.73	7170	182.30
	16	160	29.82	850.02	71.92	158.21
10.5	15	140	26.33	1.010.96	63.12	155.01
	16	140	24.77	885.25	56.58	175.95
	15	160	21.63	735.60	57.85	139.11
	16	160	22.13	609.20	45.73	186.85
21	15	140	20.15	884.90	56.86	134.80
	16	140	20.47	809.70	51.08	142.40
	15	160	17.90	584.73	48.92	133.03
	16	160	19.37	512.15	53.38	134.91

* Barrel - third zone temperature.

6.3.2.2 Expansion ratio

In general, for the treatments where pineapple pomace was added to the mixture, the results showed significant reduction in the expansion ratio when compared to the control. This reduction ranged from 8.03% to 37.56% (Table 5). The differences in the expansion can be visually observed in Fig. 1. The addition of pomace reduced the relative amount of the other components of the mixture when compared with extrusion in the absence of byproduct. According to Bullerman et al. (2008), relative reductions in the amount of starch, mainly responsible for the puffiness of the final product, had a direct impact in the expansion ratio of extruded corn product. Furthermore, this result may also have been influenced by the presence of fibres, which tend to rupture the cell walls before the gas bubbles may expanded to their full potential (LUE; HSIEH; HUFF, 1991). Decreasing in the expansion ratio by the addition of products rich in fibre was also observed in studies done by Altan, Mccarthy and Maskan (2008) and Dehghan-Shoar, Hardacre and Brennan (2010).

Figure 1 – Appearance of the extruded products

Among the treatments with the same percentage of PP, there was no significant effect ($p>0.05$) of the temperature and the moisture in the expansion of the extrudates.

Table 5 - Effects of the treatments on the characteristics of the extrudates

PP (%)	Temp (°C)	Feed moisture (%)	Expansion ratio (%)	Bulk density (g/cm ³)	WAI (g gel/g)	WSI (%)	Color			Extrudate moisture (%)	Hardness ^h (N)
							L*	a*	b*		
0	140	14	3.86±0.27 ^{ab}	0.10±0.01 ^{ef}	5.53±1.29 ^b	40.41±10.12 ^a	81.07±2.03 ^{ab}	0.99±1.62 ^{bcd}	44.53±1.82 ^{bcdef}	2.82±0.27 ^{bcd}	7.15±0.64 ^a
0	140	15	3.87±0.23 ^{ab}	0.12±0.01 ^{def}	5.85±0.56 ^{ab}	36.50±4.82 ^{ab}	83.51±1.09 ^a	-0.21±0.58 ^{cd}	45.83±0.35 ^{bcde}	3.06±0.07 ^{bc}	8.05±1.54 ^a
0	140	16	3.84±0.15 ^{ab}	0.14±0.01 ^{cde}	6.92±0.11 ^{ab}	27.99±1.01 ^{bcd}	85.43±0.32 ^a	-1.56±0.32 ^d	49.14±1.33 ^{ab}	3.91±0.06 ^a	7.75±1.13 ^a
0	160	14	4.06±0.07 ^a	0.08±0.01 ^f	6.31±0.97 ^{ab}	33.89±8.69 ^{abc}	81.74±0.43 ^{ab}	0.49±0.22 ^{bcd}	46.56±2.44 ^{abcd}	2.76±0.20 ^{bcd}	7.61±1.42 ^a
0	160	15	3.98±0.03 ^{ab}	0.10±0.01 ^{ef}	6.81±0.11 ^{ab}	29.27±2.11 ^{abcd}	83.34±0.87 ^a	-0.09±0.51 ^{dc}	46.83±1.15 ^{abc}	2.93±0.26 ^{bc}	7.55±0.14 ^a
0	160	16	3.73±0.18 ^{abc}	0.11±0.02 ^{def}	7.11±0.66 ^a	25.67±4.40 ^{bcd}	85.22±0.29 ^a	-1.05±0.44 ^d	51.35±0.54 ^a	3.30±0.19 ^{ab}	7.40±0.99 ^a
10.5	140	15	3.34±0.22 ^{cd}	0.13±0.01 ^{cde}	6.66±0.44 ^{ab}	28.28±4.99 ^{bcd}	69.14±4.31 ^c	5.88±2.64 ^a	42.54±0.88 ^{cdef}	2.60±0.31 ^{cd}	5.51±0.83 ^a
10.5	140	16	3.55±0.10 ^{bcd}	0.12±0.02 ^{def}	6.78±0.21 ^{ab}	24.86±2.22 ^{cd}	70.69±7.0 ^c	3.79±2.95 ^{ab}	41.72±4.57 ^{def}	3.04±0.44 ^{bc}	7.13±1.79 ^a
10.5	160	15	3.16±0.14 ^{de}	0.13±0.01 ^{cde}	6.83±0.03 ^{ab}	22.53±1.67 ^d	74.52±2.48 ^{bc}	3.61±1.73 ^{abc}	43.34±1.36 ^{cdef}	2.64±0.31 ^{cd}	5.99±1.28 ^a
10.5	160	16	3.14±0.13 ^{def}	0.13±0.01 ^{cde}	6.90±0.13 ^{ab}	23.14±2.99 ^{cd}	74.13±1.73 ^{bc}	2.95±1.18 ^{abc}	43.52±1.41 ^{cdef}	2.82±0.09 ^{bcd}	7.20±1.26 ^a
21	140	15	2.76±0.23 ^{efg}	0.15±0.00 ^{bcd}	6.09±0.04 ^{ab}	26.59±0.74 ^{bcd}	68.10±6.87 ^c	5.02±2.25 ^a	40.11±3.60 ^f	2.26±0.07 ^d	6.09±0.23 ^a
21	140	16	2.72±0.10 ^{fg}	0.19±0.01 ^a	6.27±0.07 ^{ab}	22.85±1.29 ^{cd}	70.12±2.66 ^c	3.98±1.48 ^{ab}	41.06±1.06 ^{ef}	2.93±0.13 ^{bc}	6.17±0.43 ^a
21	160	15	2.41±0.09 ^g	0.16±0.02 ^{abc}	6.01±0.08 ^{ab}	22.63±0.96 ^{cd}	70.12±3.69 ^c	4.93±2.03 ^a	41.20±2.32 ^{ef}	2.24±0.35 ^d	5.76±0.51 ^a
21	160	16	2.48±0.06 ^g	0.19±0.02 ^{ab}	6.01±0.11 ^{ab}	21.94±1.02 ^d	70.68±1.88 ^c	3.97±1.23 ^{ab}	39.64±1.90 ^f	2.44±0.10 ^{cd}	5.71±0.81 ^a

Mean ± Standard deviation.

Means followed by different letters are significantly different ($P<0.05$) by *Tukey's* test.

^h Data with logarithmic transformation (base 10). The means comparison was performed with transformed data and the results presented in the original scale.

6.3.2.3 Bulk density

Addition of 21% of pineapple pomace in the blends significantly increased the bulk density of the extrudates when compared with the control (Table 5). This result could be explained by the amount of fibre and sugar present in the PP. According to Dehghan-Shoar, Hardacre and Brennan (2010), these compounds have the capacity to absorb water, which can lead to products with increased density. Also, with the reduction in expansion of extrudates with fibre addition, an increase in density was expected, since more products could be compacted in a given volume. Similar effects were observed during extrusion with orange peel, grape peel, and tomato pomace (YAĞCI; GÖĞÜŞ, 2008). However, the lowest level of pomace addition (10.5%) did not affect ($p>0.05$) the product density when compared to the control.

6.3.2.4 Water absorption index (WAI) and water solubility index (WSI)

Water absorption index measures the amount of water absorbed by starch and can be used as an index of gelatinization (ANDERSON et al., 1969). The WAI for treatments without and with pomace ranged from 5.53 ± 1.29 to 7.11 ± 0.66 g gel/g and from 6.01 ± 0.08 to 6.90 ± 0.13 g gel/g (Table 5), respectively, with no significant differences ($p>0.05$) among them. The lack of difference could be explained by higher starch content in treatments without fibre addition, which swell when heated in excess water; while treatments had the fibre rich pineapple pomace, which also has the property of holding water.

When treatments without fibre addition were compared, the one with 14% of moisture and extruded at 140 °C showed the lowest WAI (5.53 ± 1.29 g gel/g), while the one with the highest moisture (16%) and temperature (160 °C) presented the highest value (7.11 ± 0.66 g gel/g). Water absorption index has been reported to be influenced by factors such as temperature and moisture. Limited water (14% moisture) could have caused more polymer damage during extrusion, resulting in low WAI. Also, according to Mercier and Feillet (1975), WAI generally increases along with the increase in extrusion temperature until a certain point (temperature peak), after which it decreases, probably due to increased dextrinization. Since in this study the increase in extrusion temperature of treatments without fibre caused an increase in the water absorption of the extrudates, no maximum WAI peak was observed in the temperature range used in this study.

Water solubility index measures the amount of soluble components released during extrusion (KIRBY et al., 1988). It is often used as an indicator of the starch degradation (dextrinization) (DING et al., 2006), since this process leads to the generation of smaller and

more water soluble molecules. Besides changes in starch, WSI may also be influenced by structural changes of other components during extrusion, such as proteins, which undergo denaturation, affecting their solubility, and fibres. According to Brennan et al. (2013a), the water solubility of dietary fibre can be increased during extrusion, indicating a potential role of this process in altering its molecular structure. However, when the WSI was evaluated, the presence of PP at both levels tested caused less solubilisation of the matrix components during extrusion when compared to the control (Table 5). This decrease in WSI may have occurred due to the fibre content of the material ($45.2 \pm 3.62\%$), which has 98.3% of it as water insoluble fraction, along with the fact that these treatments have less amount of starch in their composition, which, during extrusion, could lead to the release of more soluble compounds. A decrease in WSI with an increase in fibre was also observed by Kumar, Sarkar and Sharma (2010), who used carrot pomace.

In the treatments without pomace, there was no significant difference ($p>0.05$) in the WSI between the products extruded at 14% and 15% of moisture. However, a reduction in the WSI was observed for products extruded at 16% when compared to the control (14% moisture). According to Gomez and Aguilera (1983), the process of dextrinization is well known as the predominant mechanism of starch degradation during low moisture extrusion. Therefore, it was expected that extrusions done with lower moisture would lead to extruded products with higher WSI as a consequence of a greater intensity of dextrinization (Table 5).

6.3.2.5 Colour

Extruded products with added PP did not present any colour differences among them, but, in general, they showed a darker colour than treatments without pomace. This reduction in lightness may have been caused by the presence of PP in the final product and also due to browning reactions, such as Maillard and caramelization, promoted by the presence of sugar in the added material. Similarly, Dehghan-Shoar, Hardacre and Brennan (2010) observed reduction in lightness of extruded products when working with tomato skin.

Three of the four treatments where PP was added and the blend moisture content adjusted to 15%, presented higher a^* values when compared with the control (Table 5). The increase in redness may have been caused by browning reactions due to processing temperatures and presence of sugars in the pomace. Specifically for the treatments with 15% of moisture, this effect could have been enhanced, because Maillard reaction is favored by low moisture. Similar results were observed by Yağcı and Göğüş (2009), who verified that an increase in moisture content generally decreased the redness of the final products.

The use of pineapple pomace did not affect ($p>0.05$) the yellowness (b^* value) of the extrudates (Table 5), when results were compared to the control.

6.3.2.6 Moisture content

There was no significant difference ($p>0.05$) among the moisture content of the treatments with PP addition and the control, indicating that the drying step after extrusion (100 °C/10 min) probably homogenised the moisture content of the extruded products (Table 5). These results are important for the purpose of treatments comparison in the texture analysis.

6.3.2.7 Texture analysis

There was no difference ($p>0.05$) in hardness among treatments with and without pineapple pomace, indicating that the amount of fibre added did not affect the texture of the extruded product (Table 5). This result is in agreement with those reported by Stojceska et al. (2008), who verified that the hardness of extruded products was not related to the level of addition of cauliflower byproduct. In contrast, the study of Altan, McCarthy and Maskan (2008) showed that increasing grape pomace level resulted in an increase in the hardness of extrudates. In the present study, when extrudates obtained with the same amount of fibre but at different moisture blends and extrusion temperatures were compared, no effect ($p>0.05$) of these parameters were observed on the hardness of the product.

6.4 Conclusions

The pineapple pomace showed low fat and protein content and had dietary fibre as one of its major components ($45.22 \pm 3.62\%$), with the insoluble fraction accounting for the majority of the fibre. Low microbiological counts, water activity, and pH, along with high titratable acidity, indicated the good microbiological quality of the material and its low risk for physicochemical deterioration. The measured colour showed high L^* values, which can be a positive attribute, since dark colour may limit the application of this byproduct in foods. The pineapple pomace showed low OHC but considerable WHC, suggesting that this ingredient could be used in products that require hydration, to improve yield, and modify texture and viscosity.

Extruded products added with 10.5% and 21% of PP expanded less and were darker than the control. In general, treatments with the same amount of PP were not affected by extrusion moisture and barrel temperature. Bulk density, hardness, WAI, and b^* value of the

products added with 10.5% of PP were not different than the control, showing satisfactory results related to the properties and characteristics of extruded products, which opens the possibility of a new application for this fruit pomace.

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7 GENERAL CONCLUSIONS

This study showed that agro-industrial byproducts still have some important substances with interesting properties or activities that can be used for a second application, such as food ingredients, resulting in economic gains, lower environmental impact, thus adding value to these materials. Among the materials studied here, mango byproduct had the highest phenolic content and antioxidant activity, while pineapple byproduct showed high amount of fiber and water holding capacity. The three freeze-dried byproducts showed low water activity, pH and high acidity, important conditions to avoid the development of microorganisms in the material. Microbial counts and pesticides residues were below the limits established by the legislation, indicating that the fruit byproducts were safe for consumption. Considering the safety for food use and the amount of fiber found in the byproducts, they were explored for this potential, for both technological and nutritional aspects.

The application of the three fruit byproducts, in different concentrations, as partial fat replacers in beef burgers indicated that the pineapple byproduct was more advantageous than the others. The high fiber content present in this byproduct promoted higher water and fat retention in the burgers, improving cooking properties, which resulted in better visual characteristics (less increase in thickness and diameter reduction) and increase in yield. Furthermore, burgers with pineapple byproduct were similar to conventional burgers in the sensory attributes evaluated. Based on these results, pineapple was selected to be further studied.

The reduction and partial substitution of animal fat by pineapple byproduct and canola oil in beef burger showed positive results. Fat reduction, regardless of the use or not of the fat substitutes, led to cholesterol decrease in cooked products. Pineapple byproduct was effective in improving the cooking properties and the texture of low-fat burgers while canola oil led to the development of a healthier product, as a result of an improvement in the lipid profile, without reducing the shelf life. According to the quantitative descriptive analysis, the treatment with pineapple byproduct in association with canola oil was the closest to the conventional, indicating that the combination of these two fat replacers can be a promising alternative in the development of healthier burgers.

The use of pineapple byproduct to enhance fiber content of corn extruded products showed that the incorporation of 21% significantly altered most of the extrudates' characteristics. However, higher feasibility of use was observed when 10.5% of pineapple byproduct was added, since bulk density, hardness, WAI, and b* value of the products were

not different than the control, indicating satisfactory results related to the properties and characteristics of extruded products. These results open the possibility of a new application for this fruit byproduct.

This study showed that pineapple byproduct, currently discarded as waste in the environment, is a potential source of fiber, presenting interesting functional properties. Its application as fat replacer in beef burgers and as source of fiber in corn extruded products showed important results, from both technological and nutritional aspects. Due to this, pineapple byproduct can be considered a promising low-cost and widely available food ingredient.