University of São Paulo
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Use of fruit processing by-products for the development of cookies: physicochemical, nutritional, technological and sensory aspects

Nataly Maria Viva de Toledo

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

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Bachelor of Food Science

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I dedicate this work to my family and friends for

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"The real voyage of discovery consists not in seeking new landscapes,
but in having new eyes."

Marcel Proust

SUMMARY

RESUMO

Aproveitamento de subprodutos do processamento de frutas para desenvolvimento de cookies: aspectos físico-químicos, nutricionais, tecnológicos e sensoriais

O presente estudo visou caracterizar subprodutos de abacaxi, maçã e melão e avaliar seu potencial como ingrediente para substituição parcial de farinha de trigo em formulações de biscoitos. A adição de subprodutos de frutas contribuiu para elevar o teor de fibras dos biscoitos. O subproduto de melão se destacou por seu conteúdo mineral, enquanto que o subproduto de maçã apresentou resultados mais relevantes para compostos fenólicos e capacidade antioxidante (DPPH e ABTS). Tais comportamentos se refletiram nas formulações de biscoitos. Oito compostos fenólicos (ácido vanílico, ácido gálico, ácido sinápico, ácido salicílico, ácido p-cumárico, catequina, epicatequina e rutina) foram identificados e quantificados por HPLC tanto nos subprodutos como nos biscoitos. Com relação aos fatores antinutricionais e biodisponibilidade mineral, observou-se que a adição de subprodutos de frutas em biscoitos diminuiu os teores de fitato e oxalato e promoveu mudanças na biodisponibilidade de cálcio, ferro e zinco. Frente aos aspectos tecnológicos, observou-se que o uso de subprodutos interferiu na cor dos biscoitos, tornando-os levemente mais escuros e também enfraqueceu a rede de glúten formada, promovendo variações do diâmetro e fator de expansão. Por outro lado, observou-se que quanto maior a concentração de subprodutos, maiores foram os valores de dureza (textura), sendo as amostras consideradas mais firmes e crocantes. Do ponto de vista sensorial, verificou-se que o biscoito com 15% de subproduto de abacaxi foi o que demonstrou maior aceitação, seguido pelos cookies com 15% de subproduto de maçã, controle e 15% de subproduto de melão. Por meio de análise descritiva quantitativa (ADQ), observou-se que a adição de subprodutos de frutas alterou o perfil sensorial dos biscoitos. A partir dos resultados obtidos, concluiu-se que os subprodutos de frutas se apresentam como potenciais ingredientes para formulações de cookies, atribuindo-lhes propriedades físico-químicas, tecnológicas, nutricionais e sensoriais específicas as quais podem variar de acordo com o subproduto empregado.

Palavras-chave: Cookies; Maçã; Abacaxi; Melão; Resíduos; Mineral; Fibras; Biodisponibilidade; Fitato; Oxalato; Caco-2

ABSTRACT

Use of fruit processing by-products for the development of cookies: physicochemical, nutritional, technological and sensory aspects

The present study aimed at characterizing pineapple, apple and melon by-products and evaluating their potential as an ingredient for the partial substitution of wheat flour in cookie formulations. The addition of fruit by-products contributed to rise the cookies fiber content. Melon by-product was prominent for its mineral content, whereas apple by-product presented more relevant results for phenolic compounds and antioxidant capacity (DPPH and ABTS). Such behaviors reflected in the cookie formulations. Eight phenolic compounds (vanillic acid, gallic acid, sinapic acid, salicylic acid, *p*-coumaric acid, catechin, epicatechin and rutin) were identified and quantified by HPLC in both the by-products and cookies. Regarding the antinutritional factors and mineral bioavailability, it was observed that the addition of fruit by-products in cookies reduced the contents of phytate and oxalate and promoted changes in calcium, iron and zinc bioavailability. Facing the technological aspects, it was observed that the use of by-products interfered in the color of the cookies, making them slightly darker and also weakened the gluten formed, promoting variations in diameter and expansion factor. On the other hand, it was observed that increasing concentrations of by-products led to higher values of hardness, making the cookies firmer and crunchier. In the sensory point of view, it was verified that the cookie with 15% of pineapple by-product demonstrated the highest acceptance, followed by cookies with 15% of apple by-product, control and 15% of melon by-product. By quantitative descriptive analysis (QDA), it was found that the addition of fruit by-products altered the sensory profile of the cookies. From the results obtained, it can be concluded that the fruit by-products are potential ingredients for cookie formulation, attributing to them specific physicochemical, technological, nutritional and sensory properties which can vary according to the by-products employed.

Keywords: Cookie; Apple; Pineapple; Melon; Waste; Mineral; Fiber; Bioavailability; Phytate; Oxalate; Caco-2

1. INTRODUCTION

Besides *in natura* consumption, fruit production has been demonstrating growth, especially by the expansion of the processed food market, with emphasis for pulps, juices, canned foods, jellies, candies and minimally-processed fruits. The high demand for these products is related to the current lifestyle, which requires products of high practicality and convenience. Nevertheless, concomitant with the rise in the search for these processed foods is the increase in the generation of waste discarded by the industries, which might lead to environmental damages and represent costs for their correct treatment and disposal (Jayathilakan *et al*., 2012).

Fruit processing is considered one of the activities that most generates by-products. It is estimated that from the total mass of processed fruits, around 50% become agro-industrial waste (Gómez & Martinez, 2017). Fruit and vegetable by-products usually present significant amounts of fibers, vitamins, minerals and antioxidant compounds and, therefore, their use has been explored as alternatives in the development of new food products (Martins, Pinho & Ferreira, 2017; Pasqualone *et al*., 2017; Quiles *et al*., 21018).

Among the fruits usually found in the Brazilian market, pineapple, apple and melon can be highlighted, which are widely consumed and appreciated given their sensory characteristics; however, it is observed that their by-products still present scarce use.

Pineapple (*Ananas comosus*) is a tropical fruit, belonging to the family *Bromeliaceae*, originating from South America, including the Brazilian central zone, the northeast of Argentina and Paraguay (Sampaio, Fumis & Leonel, 2011). Among the predominant cultivars in Brazil are Pérola and Smooth Cayenne, with pineapple production in the harvest of 2017 reaching around 1.6 million tons, with emphasis for the States of Pará, Paraíba and Minas Gerais (IBGE, 2017). After pulp industrialization, pineapple central axis, crown, peel and kernel are the main by-products obtained. These residues are usually discarded or directed to the generation of alcohol, ascorbic and malic acids, animal feeds and extraction of the enzyme bromelain (digestive and anti-inflammatory agent) (Prakitchaiwattana, Boonin & Kaewklin, 2017). As applications for the pineapple by-products, studies suggest formulations of extrudates (Selani *et al*., 2014), yogurts (Sah *et al*., 2016) and beef burgers (Selani *et al*., 2016).

Apple (*Malus* spp.) is original from Asia and Europe and belongs to family *Rosaceae*. In Brazil, the most planted cultivars are Gala, Golden Delicious and Fuji, and in the harvest of 2017, the country produced approximately 1.1 million tons of the fruit (IBGE, 2017). Apple industrial processing, especially to obtain juice, generates a large amount of by-products, since bagasse (94.5%), seeds (4.4%) and kernels (1.1%) (Coelho & Wosiacki, 2010). Apple bagasse can be employed for the incorporation of phenolic compounds, pectin, citric acid and fibers in foods (Perussello *et al*., 2017)).

Original from the central region of Asia and Africa and belonging to the family *Cucurbitaceae*, melon (*Cucumis melo* L.) is one of the horticultural products that have been minimally processed in diverse forms: in the form of small balls, slices and cubes with or without peel (Muller *et al*., 2013). Rio Grande do Norte is prominent as the largest Brazilian producer, its production having reached, in 2017, around 400 thousand tons (IBGE, 2017). Among its by-products, the peels can be highlighted, mainly composed of proteins, fibers and minerals such as calcium and potassium; and the seeds, which present significant amounts of minerals such as iron, zinc, potassium, proteins and lipids, with emphasis for the linoleic and oleic acids (Storck *et al*., 2013; Petkova & Antona, 2015). Few studies report the use of melon by-products for product development. The most usual is the use of peels for the preparation of jams, glazed candies, liqueurs and jellies, whereas the seeds are consumed as snacks after drying and seasoning with salt (Miguel *et al*., 2008).

According to Martins, Pinho and Ferreira (2017), baked goods and biscuits are considered the best vehicles to increase the nutritional value of food products. This is because they are considered versatile foods, which can present distinct forms, sizes, types and flavors. Furthermore, they are affordable and include consumers of several ages and social classes. Biscuits are defined as "products obtained by the mixture of flour(s), and/or starch(es) with other ingredients, submitted to processes of kneading and cooking, either fermented or not. They might present several toppings, fillings, formats and textures" (Brasil, 2005). Their nutritional quality is often considered low for being generally formulated with only wheat flour and/or other refined flours. Thus, new formulations have been demonstrating advantages in the use of plant by-products in relation to the functional and nutritional properties of biscuits and baked goods. De Camargo *et al*. (2014) used peanut skins to increase the contents of phenolic compounds and antioxidant capacity of cookies. On the other hand, Souza *et al*. (2018) explored the bagasse of custard apple to enhance the mineral profile of cookies. Hemdane *et al*. (2015), in turn, investigated the use of by-products from wheat grinding in the preparation of breads and observed that coarse bran can contribute to rise the content of dietary fibers in the formulations.

It is known that the development of new products requires a comprehensive approach, since food matrices are complex and provide interactions among several compounds. In this sense, attention must be given to the antinutritional factors (e.g. phytates and oxalates), substances capable of interfering in nutrient bioavailability. The concept of bioavailability can be understood as the amount of nutrient that is absorbed and available for physiological functions. The bioavailability will depend of digestion, release from the food matrix, absorption by intestinal cells, and transport to body cells (Etcheverry, Grusak & Fleige, 2012). For being considered less expensive, fast and for offering better control of the variables than studies with animals or humans, *in vitro* assays are usually employed for bioavailability determination, with the use of cell cultures, such as Caco-2, being extensively employed, because of their morphological and functional characteristics that simulate absorption cells present in the small intestine mucosa (Sambuy *et al*., 2005).

Considering this perspective, the need for alternatives to use fruit by-products in the development of new products can be verified. Therefore, the present study had the purpose of characterizing fruit by-products (pineapple, apple and melon) and investigating their potential of application as ingredients in the formulations of cookies, in order to enhance their physicochemical, nutritional, technological and sensory properties.

Thereby, this thesis consists of five chapters. The first one is an introduction about the study. In the second chapter, apple, pineapple and melon by-products were characterized through physicochemical, pesticide residues and phenolic (flavonoids and phenolic acids) content, as well as the effect of the addition of fruit by-products on cookies in relation to their antioxidant capacity and phenolic profile was evaluated. In the third charpter, the cookies containing fruit by-products were characterized regarding their physicochemical, microbiological and sensory aspects. This chapter was also important to select the percentage of by-product used in the cookies. In the chapter four, the changes in te sensory profile of cookies due to the replacement of wheat flour by fruit by-products were studied. Finally, the chapter five evaluated the antinutritional factors (phytate and oxalate) present in fruit by-products and also in cookies and their influence on the bioavailability of calcium, iron and zinc.

REFERENCES

Brasil. Agência Nacional de Vigilância Sanitária. Resolução RDC nº 269, de 22 de setembro de 2005. Aprova o "Regulamento Técnico para produtos de cereais, amidos, farinhas e farelos." Diário Oficial da União. Brasília, DF, 23 de setembro de 2005.

Coelho, L. & Wosiacki, G. (2010). Sensory evaluation of bakery products with the addition of apple pomace flour. *Food Science and Technology (Campinas),* 30, 582-588.

De Camargo, A.C., Vidal, C.M.M., Canniatti-Brazaca, S.G. & Shahidi, F. (2014). Fortification of cookies with peanut skins: effect on the composition, polyphenols, antioxidant properties, and sensory quality. *Journal of Agricultural and Food Chemistry*, 62, 11228-11235.

Etcheverry, P., Grusak, M.A. & Fleige, L.E. (2012). Application of in vitro bioaccessibility and bioavailability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc, and vitamins B6, B12, D, and E. *Frontiers in Physiology*, 3, 1-22.

Gómez, M. & Martinez, M.M. (2017). Fruit and vegetable by-products as novel ingredients to improve the nutritional quality of baked goods. *Critical Reviews in Food Science and Nutrition*, 2017, 1-17.

IBGE Instituto Brasileiro de Geografia e Estatística. https://sidra.ibge.gov.br/home/lspa/brasil. Accessed 21 May 2018.

Jayathilakan, K., Sultana, K., Radhakrishna, K. & Bawa, A.S. (2012). Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review. *Journal of Food Science and Technology*, 49, 278-293.

Martins, Z.E., Pinho, O. & Ferreira, I.M.P.L.V.O. (2017). Food industry by-products used as functional ingredients of bakery products. *Trends in Food Science and Technology*, 67, 106-128.

Miguel, A.C.A., Albertini, S., Begiato, G.F., Dias, J.R.P.S. & Spoto, M.H.F. (2008). Agroindustrial use for the solids wastes deriving from minimally processed melon. *Food Science and Technology (Campinas)*, 28, 733-737.

Muller, N.G., Fasolo, D., Pinto, F.P., Bertê, R. & Muller, F.C. (2013). Phytochemical potentialities of melon (*Cucumis melo L.*) in the northwest region of Rio Grande do Sul – Brazil. *Revista Brasileira de Plantas Medicinais*, 15, 194-198.

Pasqualone, A., Laddomada, B., Alfio, S. & Giannone, V. (2017). Almond by-products: extraction and characterization of phenolic compounds and evaluation of their potential use in composite dough with wheat flour. *LWT- Food Science and Technology,* 89, 299-306.

Perussello, C., Zhang, Z., Marzochella, A. & Kumar T.B. (2017). Valorization of apple pomace by extraction of valuable compounds. *Comprehensive Reviews in Food Science and Food Safety*, 16, 776-796.

Petkova, Z. & Antova, G. (2015). Proximate composition of seeds and seed oils from melon (*Cucumis melo* L.) cultivated in Bulgaria. *Cogent Food and Agriculture*, 1: 1-15.

Prakitchaiwattana, C., Boonin, K. & Kaewklin, P. (2017). De-acidification of fresh whole pineapple juice wine by secondary malolactic fermentation with lactic acid bacteria. *International Food Research Journal*, 24, 223-231.

Quiles, A., Campbell, G.M., Struck, S., Rohm, H. & Hernando, I. (2018). Fiber from fruit pomace: a review of applications in cereal-based products. *Food Reviews International,* 34, 1-46.

Sah, B.N., Vasiljevic, T., McKechnie, S. & Donkor, O.N. (2016). Effect of pineapple waste powder on probiotic growth, antioxidant and antimutagenic activities of yogurt. *Journal of Food Science and Technology*, 53, 1698-1708.

Sambuy, Y., De Angelis, I., Ranaldi, G., Scarino, M.L., Stammati, A. & Zucco, F. (2005). The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. *Cell Biology and Toxicology*, 21, 1-26.

Sampaio, A.C., Fumis, T.F., Leonel, S. (2011). Vegetative growth and fruit characteristics of five cultivars of pineapple in the Bauru region. *Revista Brasileira de Fruticultura*, 33, 816-822.

Selani, M.M., Brazaca, S.G.C., Dias, C.T.S., Ratnayake, W.S., Flores, R.A. & Bianchini, A. (2014). Characterization and potential application of pineapple pomace in an extruded product for fibre enhancement. *Food Chemistry*, 163, 23-30.

Selani, M.M., Bianchini, A., Ratnayake, W.S., W.S., Flores, Massarioli, A.P., Alencar, S.M. & Canniatti-Brazaca, S.G. (2016). Physicochemical, Functional and Antioxidant Properties of tropical fruits co-products. *Plants Foods for Human Nutrition,* 71, 137-144.

Souza, F.T.C., Santos, E.R., Silva, J.C., Valentim, I.B., Rabelo, T.C.B., Andrade, N.R.F. & Silva, L.K.S. (2018). Production of nutritious flour from residue custard apple (*Annona squamosa* L.) for the development of new products. *Journal of Food Quality*, 2018, 1-10.

Storck, C.R., Nunes, G.L., Oliveira, B.B. & Basso, C. (2013). Folhas, talos, cascas e sementes de vegetais: composição nutricional, aproveitamento na alimentação e análise sensorial de preparações. *Ciência Rural*, 43, 537-543.

2. PINEAPPLE, APPLE AND MELON BY-PRODUCTS CAN INCREASE THE CONTENTS OF PHENOLIC ACIDS AND FLAVONOIDS IN COOKIES AND CONTRIBUTE TO THEIR ANTIOXIDANT CAPACITY

ABSTRACT

Fruit by-products usually present expressive amounts of phenolic compounds and their use has been explored in the development of new food products. This work aimed at determining the physicochemical properties of pineapple (PIB), apple (APB) and melon (MLB) by-products, besides evaluating their influence on the antioxidant capacity and phenolic compounds (phenolic acids and flavonoids) of cookies prepared with 15% of these by-products. The proximate composition showed that carbohydrates and fibers were the most relevant components in the by-products. The low values found for pH (4.19 to 5.48) and water activity (0.17 to 0.19) indicate that these by-products are not easily susceptible to deterioration. The by-products presented light color and tone close to yellow, which is desirable for their application in cookies. The APB by-product presented the highest total phenolic content (5.92 mg GAE/g) and highest antioxidant capacity by the methods DPPH and ABTS, which has reflected in the formulation of cookies. Eight phenolic compounds (vanillic acid, gallic acid, sinapic acid, salicylic acid, p-coumaric acid, catechin, epicatechin and rutin) were identified and quantified by high performance liquid chromatography (HPLC) in both the by-products and cookies. The results found indicate that fruit by-products can be applied in cookies formulations, contributing to their content of phenolic compounds and antioxidant capacity.

Keywords: Phenolic compounds; Polyphenols; Fruits; Biscuit; Waste; Residue; DPPH; ABTS

2.1. INTRODUCTION

It is estimated that after industrial processing, around 50% of the total weight of fruits and vegetables become residues, which, when inadequately discarded, entail environmental problems and operational costs. These by-products, however, usually present high nutritional value and considerable content of bioactive compounds, which are important for a good intestinal functioning, weight control, reduction in the blood cholesterol levels and a better control of the glycemic and insulin responses (Gómez & Martinez, 2017). Even with so many attributes, there are still scarce viable alternatives for the major part of plant by-products, which are usually employed as fertilizers or for animal feeding (Paraman *et al*., 2015).

Peels, bagasse, membranes and seeds are the main by-products obtained from fruit processing. It is known that the appropriate use of these by-products can minimize environmental damages, add value to products and contribute to a more straightforward and sustainable production (FAO, 2013). Fruits like apple, pineapple and melon are popularly consumed both *in natura* and processed, especially in the production of juices, pulps, jellies and craft candies. These fruits are known for their significant amounts of bioactive compounds such as fibers, vitamins and polyphenols (Sato *et al*., 2010; Storck *et al*., 2013; Selani *et al*., 2016).

Phenolic compounds are derived from the plant secondary metabolism, and act mainly as a response to ecological and physiological pressures. They are usually related to plant pigmentation and can still play an antipathogenic role. In addition to being related to the antioxidant capacity, these compounds attribute sensory characteristics to the foods, such as color, aromas, bitterness and astringency (Martins, Pinho & Ferreira, 2017). Several phenolic compounds are described in literature and, in the case of fruits, the phenolic acids and flavonoids are predominant.

The extraction of phenolic compounds from unconventional parts of foods have been explored in the last years as an alternative to identify possible natural antioxidants, such as the phenolic compounds, since their consumption is associated to beneficial effects to health because of incidence reduction and prevention of chronic degenerative diseases, such as diabetes (Sivamaruthi, Kesika & Chaiyasut, 2018), hypertension (Albuquerque *et al*., 2017), heart diseases and some types of cancers (Ambigaipalan, De Camargo & Shahidi, 2016), and obesity (Zbikowska & Kowalska, 2017).

Given their high acceptance and versatility, baked products and cookies are usually employed as sources of bioactive compounds and nutrients. Studies demonstrate that formulations enhanced with plant by-products can contribute to the rise in the content of phenolic compounds and the antioxidant capacity of these products (De Camargo *et al*., 2014; Tanska *et al*., 2016; Bhol, Lanka & Bosco, 2016). As far as we know, no literature was found focusing on the use of pineapple central axis, apple endocarp and melon peels as potential sources of antioxidant compounds in cookies formulations.

Therefore, the present work aimed at characterizing pineapple, apple and melon by-products, besides evaluating their influence on the antioxidant capacity and the phenolic acids and flavonoids of cookies prepared with 15% of these by-products. It is expected that this new approach will stimulate the use of unconventional parts of foods, thus avoiding waste, besides adding value to the by-products and functionality to cookies formulations.

2.2. MATERIAL AND METHODS

2.2.1. By-products

Pineapple (Pérola) central axis, melon peels (Yellow honeydew) and apple (Gala) endocarp were provided by Della Natural, an industry of minimally-processed foods, located in Engenheiro Coelho-SP, Brazil. In the same factory, the fruits have undergone a sanitation process with the application of chlorine dioxide (1 mL/L). The by-products were packed in plastic bags and transported at -18ºC to the Human Nutrition Laboratory of ESALQ/USP, then stored in freezer (-18°C) for a maximum of 30 days. The material was dehydrated in an E-C Modulyo freeze-dryer (Apparatus Inc., New York, USA) for 96h at -40ºC under pressure of 0.998 mbar. Subsequently, the by-products were ground in a cutting mill (Marconi, Piracicaba, Brazil), sieved at 35 mesh (Abronzinox, 0.425 mm) and stored wrapped in aluminum foil and plastic bags properly closed and frozen (-18ºC).

2.2.2. Characterization of the fruit by-products

2.2.2.1. Proximate composition

Moisture (gravimetric), ashes (gravimetric), proteins (Kjedahl, N x 6.25), and lipids (Sohxlet) were determined according to AOAC (2005). Fibers (soluble and insoluble) were determined according to the enzymatic method described by Asp, Johansson and Hallmer (1983). Available carbohydrates were obtained by difference. All analyses were performed in quadruplicate.

2.2.2.2. Physicochemical analyses

The pH was measured using a potentiometer (Quimis, Q799-D2, São Paulo, Brazil) in the samples in suspension at 10% (m/v). Water activity was verified using an Aqualab appliance (Series 4TE, Decagon devices Inc., Pullman, WA, USA) at 25ºC. Color parameters such as lightness (L), hue angle (h) and chroma (C) were read in a colorimeter (Konica Minolta, Chroma Meter, CR-400, Tokyo, Japan) with a measurement area of 8 mm in diameter, observation angle of 10ºC and standard illuminant C. For the determination of the granulometry of the flours obtained after by-product grinding, sieves from 20 to 60 mesh were used, submitted to the action of a vibrator for 15 minutes (Coelho & Wosiacki, 2010). All analyses were performed in triplicate.

2.2.2.3. Determination of pesticide residues

Analysis of pesticide multiresidue determination was performed in the fruit by-product samples by ultra-high-pressure liquid chromatography and gas chromatography. The analysis was performed by the company Agrosafety (Piracicaba, SP, Brazil), according to ABNT NBR ISO/IEC 17025. The pesticides investigated and their Maximum Residue Limits (MRL) were stipulated according to the recommendations of the authorized monographs bank found in the Brazilian Health Regulatory Agency (ANVISA) and the Program for the Analysis of Pesticide Residues in Foods (PARA) (Brasil, 2015).

2.2.3. Cookies preparation

For the preparation of the cookies, formulations developed by Toledo *et al.* (2017) were adopted. Initially, 105 g of sugar were mixed with 75 g of butter in a planetary mixer (Philips Walita, RI7915, China) for 10 s. Subsequently, 50 g of eggs were added and the mixture was stirred for further 5 s. Then, 170 g of wheat flour and 30 g of by-products (15% of substitution of wheat flour by by-product flour) were added. In the case of the control cookie, it was prepared with only wheat flour (200 g). Finally, 10 g of baking powder and 25 g of chocolate were added and mixed for 10 s. The cookies were cut with a circular mold and baked in an electric oven (Perfecta, VIPAO Coral 4, Curitiba, Brazil) at 180ºC for 10 minutes. After cooling, the cookies were stored in plastic film and maintained at room temperature (25ºC) until the moment of the analyses. Four formulations were prepared, which comprised the control (CCT) and the cookies with 15% of fruit by-products: pineapple (CPI), apple (CAP) and melon (CML). The procedure was repeated twice to obtain the batches.

The wheat flour type 1 with 10% of protein (Dona Benta), refined sugar (União), baking powder (Royal), eggs (Qualitá), salted butter (Scala) and pieces of semisweet chocolate bar with 40% of cocoa (Nestlé) used in cookie preparation were obtained from the local market in Piracicaba, SP, Brazil.

2.2.4. Total phenolics and antioxidant capacity

2.2.4.1. Extraction

Extracts were prepared from both the fruit by-products and the cookies. The fruit by-products were used in their dehydrated form. On the other hand, the cookies were ground using mortar and pistil and were sieved (Abronzinox, 1 mm). For being samples rich in lipids, the cookies were previously degreased with hexane (m/v, 1:5). The solution was kept under stirring for 5 minutes and the process was repeated three times. Subsequently, the solvent was discarded and the precipitate was placed on filter paper and maintained in a laminar flow cabinet until drying (De Camargo *et al.*, 2014). The material was immediately used for the process of extraction. The extracts of the by-products and cookies were prepared as described by Bloor (2001). Then, 1g of sample was weighed and 10 mL of the solvent composed of ethanol:water (80:20 v/v) was added. The mixture was subjected to ultrasonic bath (Ultra Cleaner, Unique, Indaiatuba, Brazil) at 25ºC for 20 minutes. The material was centrifuged (Nova Técnica NT 825, Piracicaba, Brazil) at 4000 rpm for 15 minutes, with the supernatant being used for the analyses of total phenolics, antioxidant capacity and determination of phenolic acids and flavonoids.

2.2.4.2. Total phenolic content

The total phenolic content was determined according to the spectrophotometric method described by Singleton, Orthofer and Lamuela (1999). The analyses were conducted in triplicate and the results were expressed in mg gallic acid equivalent (GAE)/g sample.

2.2.4.3. Antioxidant capacity

The antioxidant capacity was evaluated by DPPH assays, according to Brand-Williams, Cuvelier and Berset (1995), and by ABTS assays, following the methodology described by Re *et al.* (1999). In both methods, the analyses were conducted in triplicates and Trolox was used as reference standard. The results were expressed in μmol of Trolox/g sample.

2.2.5. Identification and quantification of the phenolic acids and flavonoids by HPLC

The quantitative and qualitative determination of the phenolic acids and flavonoids was performed according to He *et al.* (2011) with adaptations. A high performance liquid chromatography (HPLC) (Shimadzu 20A, Kyoto, Japan) was used, equipped with a pumping system model LC-20AT, automatic sample injector model SIL-20AHT, column oven model CTO-20A, communicator model CBM-20A and UV detector (280 and 370 nm) model SPD-20A. A C18 column (Waters Spherisorb ODS2; 4.6 x 250 mm, 5 μm) was employed for the separation at 40°C maintained by the column oven. The mobile phases used were A (1% of formic acid in aqueous solution) and B (100% of methanol), which were eluted in a linear gradient: solvent A from 100 to 40% in 45 minutes and from 40 to 0% in 5 minutes, returning to 100% in 10 minutes and lastly maintained at 100 % for 5 more minutes. The flow used was of 0.7 mL/min. The identification of the phenolic compounds was confirmed by the comparison of their retention times and UV/visible spectrum with those of the authentic materials. For quantification, the calibration curves were made from authentic standards (Sigma-Aldrich, St Louis, MO, USA). To validate the method, the linearity, limits of detection (LOD) and quantification (LOQ) were determined.

2.2.6. Statistical analyses

A completely randomized design was used. The data were subjected to analysis of variance (ANOVA) for the F test and the comparison of means by Tukey's test (p<0.05). The analyses were performed by the software Statistical Analysis System (SAS) version 9.2.

2.3. RESULTS AND DISCUSSION

2.3.1. Characterization of the fruit by-products

The fruit by-products were prepared in the form of flour to be used on the cookie's formulation. Tests to evaluate the best drying method were perfomed in a forced air circulation oven (60ºC) for 24h and by freeze drying at -40ºC. The oven drying method, although considered less expensive, was not efficient since the samples became dark and sticky (Figure 1a) due to the occurrence of the Maillard reaction (i.e. a non-enzymatic browning reaction that may occur during thermal processing and/or prolonged storage of foods containing proteins and reducing sugars) (Tamanna & Mahmood, 2015). On the other hand, the lyophilization showed uniformly dried samples after 96h drying (Figure 1b). It is known that this is one of the most suitable drying methods to preserve the flavor and aroma of products since it presents insignificant losses of volatile compounds and enzymes (Miteva *et al*., 2008). Therefore, the lyophilization process was chosen to dry the fruit by-products.

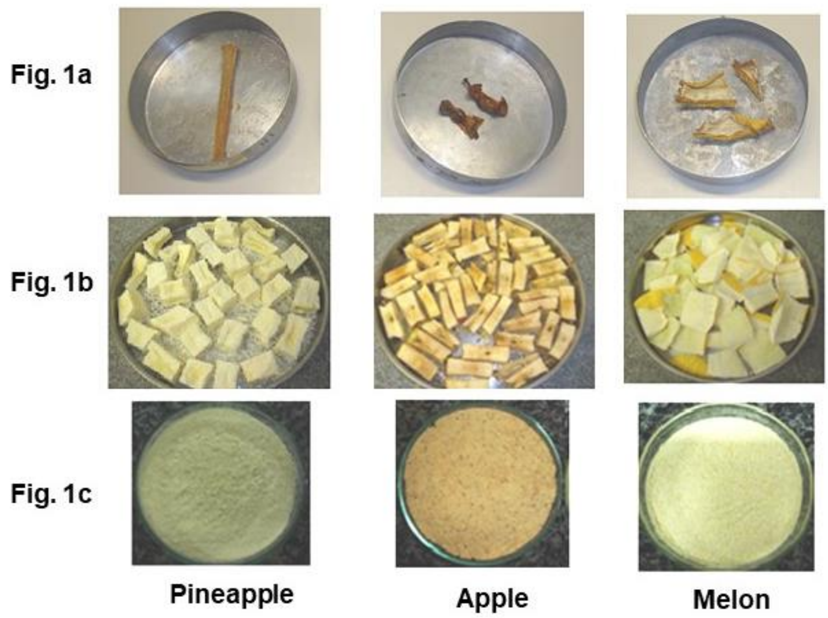

Figure 1a. Pineapple central axis, apple endocarp and melon peels dried in an oven at 60ºC for 24h.
Figure 1b. Pineapple central axis, apple endocarp and melon peels lyophilized at -40ºC for 96h.
Figure 1c. Pineapple, apple and melon flours

2.3.1.1. Proximate composition

Carbohydrates were the major macronutrients identified in the composition of the by-products of pineapple (PIB) and of apple (APB), with melon by-product (MLB) presenting the lowest content of this nutrient (Table 1). The values found for PIB and APB were superior to those observed by studies that have contemplated the use of the lyophilized by-products, which registered 43.46% (pineapple peel and bagasse) and 39.35% (apple bagasse) of carbohydrates (Selani *et al*., 2014; Sato *et al.*, 2010). This variation in the carbohydrate contents can be explained because in the present study, PIB was composed of only the central axis and APB, of the endocarp. Regarding MLB, values between 20 and 45% were reported in the literature for melon peels (Gondim *et al*., 2005; Storck *et al.*, 2013), which are consistent with the present study.

Table 1. Proximate composition of the pineapple (PIB), apple (APB) and melon (MLB) by-products

	PIB	APB	MLB
Moisture	6.48±0.03[1a2]	4.69±0.14[b]	4.42±0.23[b]
Ash	1.11±0.10[c]	2.95±0.1[b]	10.64±0.5[a]
Lipid	0.54±0.02[c]	1.33±0.14[b]	1.76±0.05[a]
Protein	3.23±0.13[a]	2.91±0.13[b]	3.11±0.16[ab]
Carbohydrate	74.01	68.62	33.77
Total Dietary Fiber	14.63±0.3[a]	19.50±0.31[b]	46.30±2.49[c]
Insoluble Dietary Fiber	13.55±0.31[b]	15.73±0.21[b]	43.53±2.64[a]
Soluble Dietary Fiber	1.08±0.08[c]	3.77±0.27[a]	2.77±0.21[b]

[1]Mean±standard deviation (*n*=4). [2]Different letters in the same row indicate significant difference ($p<0.05$) the among by-products. Results are expressed as g/100g of fresh weight basis.

The fibers had great representativity in the fruit by-products, with the highest contents registered for MLB (46.30%), which was already expected since peels usually present more significant amounts of fiber (Morais *et al.*, 2017). In general, the insoluble fibers stood out in relation to the soluble ones in all treatments. Insoluble fibers are essential to the regulation of the intestinal movements and help in the prevention of constipation (Almaraz *et al.*, 2015). Regarding the content of soluble fibers, APB was prominent compared to the other samples, possibly because of the high pectin content found in apple bagasse, which is one of the main raw materials for the commercial extraction of this polysaccharide (Willats, Knox & Mikkelsen, 2006). The soluble fibers present an important physiological function, since they positively affect the metabolism of available carbohydrates and lipids, promoting the reduction in the absorption of these nutrients in the human intestine. The proper consumption of fibers is still related to the reduction in the risk of chronic diseases, such as obesity, cardiovascular diseases, chronic diseases of the kidneys and diabetes (Fujii *et al.*, 2013). In the case of PIB, it was observed that the contents of total fibers found in the fruit kernel (14.63%) were inferior to those reported in the peels (Leonel, Leonel & Sampaio, 2014). Conversely, the fiber contents in melon peels (46.30%) was similar to that demonstrated by Storck *et al.* (2013). In relation to APB, in a study using the fruit bagasse, Coelho and Wosiacki (2010) reported a superior amount (43.02%) of fibers in comparison to the present study. This fact may be related to the presence of peels in apple bagasse, which contributes to the superior contents of fibers (Morais *et al.*, 2017).

About the moisture, PIB presented the highest value in comparison to the others. In general, superior values were observed in the literature (except for pineapple), with moisture varying between 3.77% and 11.85% for pineapple by-products (bagasse and peels) (Selani *et al.*, 2014; Leonel, Leonel & Sampaio, 2014), 7.10% to 13.72% for apple bagasse (Coelho & Wosiacki, 2010; Sato *et al.*, 2010) and 6.77% to 10.2% for melon peels (Gondim *et al.*, 2005; Storck *et al.*, 2013). It is known that moisture values may vary depending on the drying method, time of exposure to the drying process and storage conditions, since after dehydration processes the products become highly hygroscopic, potentially absorbing significant amounts of water.

For the ash, there was a significant difference ($p<0.05$) among all treatments. The MLB presented ash content ten times superior to PIB, representing the highest and lowest ash contents registered, respectively. Comparing to the literature, values of 11.66% were detected for the ash of melon by-products (Storck *et al.*, 2013), between 2.24% and 4.70% (Selani *et al*.,2014; Gondim *et al.*, 2005) for pineapple and values from 1.46% to 2% were observed for apple (Coelho & Wosiacki, 2010; Sato *et al.*, 2010). Factors such as cultivar, soil, climate conditions and fertilization might infer in the minerals available in the foods and, consequently, in their ash contents (Davis, Epp & Riordan, 2004).

Proteins and lipids were the least representative macronutrients in the by-products. Values superior to those found for proteins in the present study were reported for pineapple by-products (4.71% to 6.63%) (Selani *et al.*, 2014; Gondim *et al.*, 2005) and for melon peels (9.07% to 19.90%) (Al-Sayed & Ahmed, 2013; Storck *et al.*, 2013), whereas for apple, values from 2.42% to 3.35% (Sato *et al.*, 2010; Coelho & Wosiacki, 2010) were registered, which are coherent with this research. On the other hand, for lipids, the results found endorse the literature, with contents of 0.61% observed for pineapple bagasse (Selani *et al.*, 2014); 1.06% to 2.14% for apple bagasse (Sato *et al.*, 2010) and 1.58% to 2.45% for melon peels (Al-Sayed & Ahmed, 2013; Storck *et al.*, 2013). The low lipid contents presented were expected, since the by-products analyzed did not present seeds in their composition, which usually are the main source of fatty acids in plant-derived products (Morais *et al.*, 2017).

2.3.1.2. Physicochemical analyses

Comparing the different by-products, a significant difference ($p<0.05$) was observed among the pH values of all treatments (Table 2), with PIB and MLB exhibiting the lowest and highest values, respectively. Other studies that have used the edible parts of the fruits reported for pineapple pH values between 4.07 and 4.38 (Pereira *et al.*, 2009); for apple, 3.70 to 3.88 (Fontes *et al.*, 2008) and for melon, values between 5.01 and 6.95 (Aroucha *et al.*, 2007). Such variations in sample pH are considered acceptable, since this parameter is associated to the process of fruit ripening and might present alterations according to the point of harvest (Pereira *et al.*, 2009).

Table 2. pH, water activity (a_w), color parameters (L, C, h) referring to the fruit by-products

	PIB	APB	MLB
pH	4.19±0.01[c]	4.37±0.06[b]	5.48±0.00[a]
a_w	0.19±0.00[a]	0.19±0.00[a]	0.17±0.00[b]
L	90.47±0.60[a]	77.35±0.78[c]	88.44±0.48[b]
C	18.90±0.44[b]	27.00±0.52[a]	18.72±0.80[b]
H	97.85±0.31[a]	86.97±0.68[c]	94.57±1.03[b]

[1]Mean±standard deviation (n=3). [2]Different letters in the same row indicate significant difference (p<0.05) among the by-products. PIB: pineapple by-product; APB: apple by-product; MLB: melon by-product. L: lightness; C: chroma; h: hue angle.

With respect to the water activity, it was observed that MLB was the by-product that obtained the lowest value (0.17), significantly differing (p<0.05) from the others. The results found are consistent with the study of Selani *et al.* (2014), who registered the value of 0.14 for pineapple bagasse, but inferior to those reported by Coelho and Wosiacki (2010) for apple bagasse (0.81). Proper handling, storage and use of specific packages are relevant factors for the maintenance of the low levels of water activity of the dehydrated by-products, in order to avoid the deterioration of the material by microorganisms and enzymatic activities.

Given that plant by-products can be used as ingredients in formulations of new products, it is important to consider their color parameters, since this attribute is directly related to consumer's acceptance (Selani *et al.*, 2016). All by-products studied presented elevated lightness values (L), with emphasis for PIB, indicating that this by-product is lighter than the others. This is an important and positive result, since the ingredients with dark color present higher limitations for application in foods. No significant differences (p>0.05) were observed between PIB and MLB for chroma (C). The APB presented the highest C value and, consequently, was considered the one with the highest purity and color intensity. Considering the values of the hue (h) angle (from 86.97º to 97.85º), it was proven that the by-products presented tones closer to yellow, with a significant difference (p<0.05) among all treatments.

One of the main applications suggested for the use of fruit by-products is the preparation of baked goods and cookies. For these products, the particle size of the flour used consists in another relevant quality attribute, since particle size interferes in water absorption capacity, in the time of mixing and in sensory characteristics such as texture, flavor and visual aspect (Abera, Solomon & Bultosa, 2017). It was observed that the particle size of the flours obtained from the fruit by-products was mostly inferior to 250 µm (Table 3), which refers to a flour with fine and small particles. The flour obtained from PIB was considered the one with the lowest granulometry and most homogeneous composition. There was no significant difference (p>0.05) of final yield (particles < 250 µm) between the flours of APB and MLB. Coelho and Wosiacki (2010) developed a flour from apple bagasse and reported granulometry inferior to 500 µm for around 60% of the product. Selani *et al.* (2016) found granulometry inferior to 211 µm for 42% of flour obtained from pineapple peels and bagasse. Particle size may vary according to the fruit by-products, methods used for their preparation and considering the destination and application of the material.

Table 3. Size distribution of the particles of pineapple (PIB), apple (APB) and melon (MLB) freeze-dried by-products

Size (μm)	**% Oversize particles[1]**		
	PIB	**APB**	**MLB**
841	8.86±0.43[2][a][3]	5.08±0.04[b]	3.94±0.18[c]
500	12.50±0.24[c]	18.61±0.70[b]	21.29±0.34[a]
350	3.81±0.08[c]	5.03±0.22[b]	7.40±0.51[a]
300	6.67±0.36[c]	8.33±0.09[b]	10.10±0.26[a]
250	3.69±0.13[b]	9.95±0.47[a]	4.06±0.10[b]
< 250	64.47±0.81[a]	53.00±0.66[b]	53.21±0.49[b]

[1]Percentage of particles with diameter over the size (μm) described in the first column. [2]Mean±standard deviation (*n*=3). [3]Different letters in the same row indicate significant difference (p<0.05) among the by-products. PIB: pineapple by-product; APB: apple by-product; MLB: melon by-product.

2.3.1.3. Determination of pesticide residues

For the use of unconventional fruit parts in the human diet, investigating their levels of residual pesticides becomes mandatory, because these compounds might trigger harmful effects to health, such as the development of dermatitis to some types of cancers (Dennis *et al.*, 2010). According to the results, pesticide residues were not detected for PIB. Differently, 0.092 mg/kg of phosmet was verified in APB and 0.069 mg/kg of the active ingredient imazalil was detected in MLB. However, despite the presence of these pesticides in the by-products, it was verified that all of them were in accordance with the levels recommended by the current legislation, since the MRLs of both pesticides is equal to 1.0 mg/kg for these fruits (Brasil, 2015).

2.3.2. Total phenolic contents and antioxidant capacity of by-products and cookies

Among the by-products, the APB extract presented the highest value for total phenolic content, followed by MLB and PIB. It is worth emphasizing that apple endocarp demonstrated content of total phenolics even superior to other by-products cited in the literature (mg GAE/g), such as passion fruit peel and seed (3.43) and mango bagasse (4.50) (Infante *et al.*, 2013). Besides differences between species and cultivars, the content of phenolic compounds might vary according to the part of the fruit used for extraction. Leaves and peels, being more exposed to environmental damages than fruit pulp, require more protection against pathogens, leading the production of secondary metabolites, such as phenolic compounds, to be higher in these parts (Packer *et al*., 2015). Melon peels, for instance, presented total phenolics content of 4.58 mg GAE/g, which was consistent with the study of Mallek-Ayadi, Bahloul and Kechaou (2016), but it was inferior to the reported by Zeb (2016) for the fruit seeds. Regarding PIB by-product, it was observed that the results found corroborate with data of Infante *et al.* (2013) and Selani *et al.* (2016), which have reported total phenolics (mg GAE/g) between 2.40 and 3.78 for pineapple peels and bagasse.

Table 4. Total phenolic contents and antioxidant capacity (DPPH and ABTS) of the fruit by-products and cookies

	Total phenolic content (mg GAE/g)	**DPPH (µmol Trolox/g)**	**ABTS (µmol Trolox/g)**
PIB	2.82±0.60[1c2]	7.14±0.34[c]	7.24±0.16[c]
APB	5.92±1.78[a]	23.83±0.38[a]	19.87±0.44[a]
MLB	4.58±1.31[b]	16.60±0.23[b]	15.48±0.12[b]
CCT	7.84 ± 0.05[c]	3.94 ± 0.70[c]	5.39 ± 0.33[c]
CPI	7.80 ± 0.13[c]	5.82 ± 0.11[b]	7.17 ± 0.33[b]
CAP	16.91 ± 0.19[a]	11.97 ± 0.25[a]	12.35 ± 0.83[a]
CML	11.26 ± 0.23[b]	11.98 ± 0.46[a]	12.84 ± 0.49[a]

[1]Mean±standard deviation (*n*=3). [2]Different letters in the same column indicate significant difference (*p*<0.05) between the same group of treatments (by-products or cookies). PIB: pineapple by-product; APB: apple by-product; MLB: melon by-product; CCT: cookie control; CPI: cookies containing 15% of pineapple by-product; CAP: cookies containing 15% of apple by-product; CML: cookies containing 15% of melon by-product. Results are expressed on dry weight basis.

Cookies are traditionally prepared with ingredients such as wheat flour and chocolate, which contain polyphenols, mainly phenolic acids (cereals) and flavonoids (cocoa) (Giordano *et al.*, 2017; Ramos, Martín & Goya, 2017). Nevertheless, traditional cookies usually do not present functional properties or added nutritional value. In this sense, the incorporation of plant by-products in the development of baked goods has been faced as a strategy to carry bioactive compounds. The use of fruit by-products in the formulation of cookies contributed to the rise of more than 100% in the content of phenolic compounds (from 7.80 up to 16.91 mg GAE/g), with the treatment that employed apple endocarp (CAP) considered the most promising. Aksoylu, Çagindi and Kose (2015) also developed cookies, but with poppy and grape seeds and detected 8.44 and 17.90 (mg GAE/g) of total phenolics, respectively. Pathak *et al.* (2016) included mango peel flour in whole bread formulations and observed a rise from 2.20 to 7.57 in the content of total phenolics (mg GAE/g). Likewise, Bhol, Lanka and Bosco (2016) employed pomegranate bagasse for the development of breads and obtained a total of phenolics five times superior to the control.

The antioxidant capacity of the compounds present in the foods is related to the chemical structure, which is responsible for redox properties, and which develop a fundamental role in the adsorption and neutralization of the reactive oxygen species (ROS), eliminating the singlet and triplet oxygen, or decomposing the peroxides (Cartea *et al.*, 2011). Similarly, to the content of total phenolics, there was also a significant difference (p<0.05) among the antioxidant capacity of the by-products, especially APB. In general, the incorporation of fruit by-products in cookies increased their antioxidant capacity (Table 4), as demonstrated by the results obtained by DPPH and ABTS assays.

Despite being considered direct methods, it was observed that by the DPPH method, the values of antioxidant capacity (µmol of Trolox/g) varied between 7.14 and 23.83 for the by-products and 3.94 to 11.98 for the cookies, whereas with the ABTS method, results from 7.24 to 19.87 and between 5.39 and 12.84 were observed for by-products and cookies, respectively. This variation in the results is attributed to the distinct way for evaluation of the antioxidant capacity of each method, besides being distinguished in relation to the solubility of the compounds identified: by ABTS assays, both the lipophilic and hydrophilic compounds can be identified, whereas by DPPH assays, there is a higher sensitivity for the hydrophilic compounds (Müller, Fröhlich & Böhm. 2011). For both methods, it was observed that the antioxidant capacity of the by-products was superior to the cookies, which was expected since the fruit by-products usually present more complex profiles and more expressive amounts of phenolics than the products based on cereals (Gómez & Martinez, 2017; Giordano *et al.*, 2017). In the case of baked goods and cookies, the baking step can help in the release of conjugated phenolic acids, contributing to the content of total phenolic compounds and antioxidant capacity. Other reactions such as Maillard, caramelization and oxidation of phenols may contribute to the rise in total phenolic compounds in foods (Ragaee, Seetharaman & Abdel Aal, 2014).

2.3.3. Phenolic acids and flavonoids

Phenolic acids and flavonoids are considered the most abundant phenolic compounds in foods. Considering the phenolic compounds identified, fruit by-products APB and MLB presented respectively 7 and 5 phenolic compounds among the 8 analyzed. The MLB was prominent, especially for the presence of sinapic acid and salicylic acid (Table 5). Among the flavonoids, rutin was the compound of major relevance in this by-product. Other compounds, such as vanillic acid and catechin, were also identified, but in lower amounts when compared to the other by-products. Mariod and Matthaus (2008) also identified expressive amounts of sinapic acid, vanillic acid and catechins but in melon seeds. Rolim *et al.* (2018) demonstrated that there are differences among melon by-products, with a higher occurrence of phenolics in the peels than in the seeds, with a prevalence of gallic acid, salicylic acid and catechin in the first, whereas in the seeds, higher concentrations of vanillic acid, salicylic acid and catechin were observed.

Table 5. Phenolic acids and flavonoids identified in fruit by-products and cookies

	By-products			Cookies				Validation parameters		
	PIB	APB	MLB	CCT	CPI	CAP	CML	LOD (µg)	LOQ (µg)	R^2
Vanillic acid	15.42±0.46[1b2]	928.21±0.70[a]	9.02±0.27[c]	2.18±0.09[c]	2.76±0.11[b]	74.49±0.08[a]	1.95±0.02[d]	0.09	0.29	0.999
Gallic acid	ND	9.22±0.05[a]	ND	118.30±1.22[c]	147.01±7.56[a]	129.30±024[b]	130.45±7.35[b]	0.10	0.30	0.998
***p*-coumaric acid**	23.06±0.42[a]	ND	ND	ND	ND	ND	ND	0.01	0.03	0.996
Salicylic acid	13.53±1.32[b]	Tr	238.73±1.23[a]	3.20±0.11[b]	ND	ND	10.04±0.78[a]	0.07	0.22	0.999
Sinapic acid	Tr	50.14±0.13[b]	988.98±4.17[a]	220.06±7.71[b]	120.09±5.52[c]	97.86±0.87[d]	265.97±7.69[a]	0.21	0.65	0.995
Catechin	10.30±0.03[1c2]	91.69±0.35[a]	20.15±0.73[b]	12.63±0.52[c]	17.19±3.92[b]	26.60±0.01[a]	19.06±1.82[b]	0.01	0.02	0.998
Epicatechin	ND	1.051.49±1.45[a]	ND	6.51±0.35[d]	15.77±1.82[c]	34.05±0.22[a]	19.87±1.40[b]	0.22	0.67	0.992
Rutin	ND	24.28±0.72[b]	272.70±1.41[a]	ND	ND	1.68±0.10[b]	14.94±1.18[a]	0.23	0.69	0.994

[1]Mean±standard deviation (*n*=3). [2]Different letters in the same row indicate significant difference (p<0.05) between the same group of treatments (by-products or cookies). ND: not detected; Tr: traces; PIB: pineapple by-product; APB: apple by-product; MLB: melon by-product; CCT: cookie control; CPI: cookies containing 15% of pineapple by-product; CAP: cookies containing 15% of apple by-product; CML: cookies containing 15% of melon by-product; LOD: limit of detection; LOQ: limit of quantification. Results expressed as µg/g of dry basis.

Apple by-product (APB), which represented a more expressive result for total phenolic content and antioxidant capacity, also demonstrated (Table 5) high amounts of epicatechin and vanillic acid and, in lower concentrations, catechin, sinapic acid, rutin and gallic acid. Literature indicates that phenolic acids, such as salicylic, gallic, propylgalate and sinapic are frequent in apple bagasse (Soares *et al.,* 2008), whereas in the peels, there is predominance of flavonoids such as quercetin, catechin, epicatechin and procyanidins, besides chlorogenic acid (Assumpção *et al.*, 2018).

On the other hand, pineapple by-product (PIB) presented the least significant amounts of phenolic compounds, with *p*-coumaric acid as the most relevant compound, followed by vanillic and salicylic acids. Regarding flavonoids, only catechin was identified. According to Wen and Wrolstad (2002) and Bataglion *et al.* (2015), in pineapple fruit there are phenolic acids such as: *p*-coumaric, ferulic, sinapic, caffeic, syringic and *p*-hydroxybenzoic, whereas among the flavonoids, kaempferol and quercetin are the most significant.

For the cookies, it was observed that the addition of fruit by-products significantly increased the concentrations of the phenolic compounds analyzed in relation to the control, except for the salicylic acid in CPI and CAP (Table 5). The control (CCT), when compared to CPI and CAP, presented higher concentration of only sinapic acid. Conversely, CML demonstrated values of sinapic and salicylic acids 120% and 313% superior to CCT, respectively, in addition of significant amounts of catechin, epicatechin and rutin. It is worth mentioning that wheat flour, the main ingredient of the cookies, also presents relevant amounts of phenolic compounds, with emphasis in the ferulic, sinapic, *p*-coumaric, syringic and vanillic acids (Gotti *et al.*, 2018).

For CAP, relevant concentrations of vanillic acid, catechin an epicatechin were verified, whereas for CPI, there was prevalence of gallic acid, followed by sinapic acid, vanillic acid, epicatechin and catechin. Hidalgo *et al.* (2018), studying formulations of biscuits prepared with only wheat flour and water, identified the predominance of ferulic acid, followed by *p*-coumaric, vanillic and *p*-hydroxybenzoic acids in their formulation. According to Ragaee, Seetharaman and Abdel-Aal (2014), baking might also affect the proportion of various phenolic compounds because of thermal degradation (e.g. vanillin and vanillic acid can be produced by the decomposition of ferulic acid). Furthermore, the authors affirm that the oven might favor the release of some phenolic acids, such as syringic, ferulic, vanillic and *p*-coumaric or simple phenolics from wheat flour because of the degradation of conjugated compounds, such as tannins.

2.4. CONCLUSIONS

Regarding fruit by-products, carbohydrates and fibers were the components present in the highest amounts, whereas proteins and lipids presented less representative values. It was verified that all by-products were within the normative standards stipulated for pesticide residues, demonstrating that the application of these by-products in food products did not represent health risks regarding this aspect. The low values for pH and water activity reported contribute to the low risk of by-product deterioration by microbial or enzymatic activity. Furthermore, all by-products presented light color and tone close to yellow, which is desirable, since the ingredients with dark color might limit the application in cookies.

The partial replacement of wheat flour by fruit by-products in cookies positively influenced the content of total phenolics, antioxidant capacity and the concentration of phenolic acids and flavonoids. The melon and apple by-products presented more significant amounts of phenolic compounds when compared to the pineapple by-product, which reflected in the cookies formulations. Therefore, the use of fruit by-products can be indicated to enhance the quality of cookies with respect to the presence of phenolic compounds and antioxidant capacity.

REFERENCES

Abera, G., Solomon, W.K. & Bultosa, G. (2017). Effect of drying methods and blending ratios on dough rheological properties, physical and sensory properties of wheat–taro flour composite bread. *Food Science & Nutrition*, 5, 653-661.

Albuquerque, J.G.F, Assis, V.L, Almeida, A.J.P.O., Basilio, I.J.L.D., Luciano, M.N., Meireles, B.R.L.A., Cordeiro, A.M.T.M., Araújo, I.G.A., Veras, R.C., Ribeiro, T.P. & Medeiros, I.A. (2017). Antioxidant and vasorelaxant activities induced by northestern Brazilian fermented grape skins. *BCM Complementary and Alternative Medicine*, 17, 1-8.

Almaraz, R.S., Fuentes, M.M., Milla, S.P., Plaza, B.L., Lopez, L.M.B. & Candela, C.G. (2015). Fiber-type indication among different pathologies. *Nutricion Hospitalaria,* 31,2372-2383.

Al-Sayed, H.M.A. & Ahmed, A.R. (2013). Utilization of watermelon rinds and sharlyn melon peels as a natural source of dietary fiber and antioxidants in cake. *Annals of Agricultural Science*, 58, 83–95.

Ambigaipalan, P., de Camargo, A.C. & Shahidi, F. (2016). Phenolic compounds of pomegranate byproducts (outer skin, mesocarp, divider membrane) and their antioxidant activities. *Journal of Agricultural and Food Chemistry*, 64, 6584-6604.

AOAC Association of Official Analytical Chemists. 2005. Official Methods of Analysis of AOAC International. 18th ed. Gaithersburg: AOAC International.

Aroucha, E.M.M., Morais, F.A., Nunes, G.H.S., Tomaz, H.V.Q., Sousa, A.E.D. & Neto, F.B. (2007). Caracterização física e química de melão durante o seu desenvolvimento. *Revista Brasileira de Fruticultura,* 29, 296-301.

Aksoylu, Z., Çagindi, O. & Kose, E. (2015). Effects of blueberry, grape seed powder and poppy seed incorporation on physicochemical and sensory properties of biscuit. *Journal of Food Quality*, 38,164-174.

Asp, N. G., Johansson, C. G. & Hallmer, H. (1983). Rapid enzimatic assay of insoluble and soluble dietary fiber. *Journal of Agricultural and Food Chemistry*, 31, 476-482.

Assumpção, C.F., Hermes, V.S., Pagno, C., Castagna, A., Mannucci, A., Sgherri, C., Pinzino, C., Ranieri, A., Flôres, S.H. & Rios, A.O. (2018). Phenolic enrichment in apple skin following post-harvest fruit UV-B treatment*. Postharvest Biology and Technology*, 138, 37-45.

Bataglion, G.A., Da Silva, F.M.A., Eberlin, M.N. & Koolen, H.H.F. (2015). Determination of the phenolic composition from Brazilian tropical fruits by UHPLC-MS/MS. *Food Chemistry*, 180, 280-287.

Bhol, S., Lanka, D. & Bosco, S.J.D. (2016). Quality characteristics and antioxidant properties of breads incorporated with pomegranate whole fruit bagasse. *Journal of Food Science and Technology*, 53, 1717-1721.

Bloor, S. J. (2001). Overview of methods for analysis and identification of flavonoids. *Methods in Enzymology*, 335, 3-14.

Brand-Williams, W., Cuvelier, M. E. & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28, 25–30.

Brasil. Agência Nacional de Vigilância Sanitária. (2015). *Monografias Autorizadas - Programa de Análise de Resíduos de Agrotóxicos em Alimentos (PARA). Relatório 2013-2015.* http://portal.anvisa.gov.br/programa-de-analise-de-registro-de-agrotoxicos-para. Accessed 05 May 2018.

Cartea, M.E., Francisco, M., Soengas, P. & Velasco, P. (2011). Phenolic compounds in Brassica vegetables. *Molecules*, 16, 251-280.

Coelho, L. & Wosiacki, G. (2010). Sensory evaluation of bakery products with the addition of apple pomace flour. *Food Science and Technology (Campinas),* 30, 582-588.

Davis, D.R., Epp, M.D. & Riordan, H.D. (2004). Changes in USDA food composition data for 43 garden crops, 1950 to 1999. *Journal of the American College of Nutrition,* 23, 669-682.

De Camargo, A.C., Vidal, C.M.M., Canniatti-Brazaca, S.G. & Shahidi, F. (2014). Fortification of cookies with peanut skins: effect on the composition, polyphenols, antioxidant properties, and sensory quality. *Journal of Agricultural and Food Chemistry*, 62, 11228-11235.

Dennis, L. K., Lynch, C. F., Sandler, D. P. & Alavanja, M. C. R. (2010). Pesticide Use and cutaneous melanoma in pesticide applicators in the agricultural heath Study. *Environmental Health Perspectives*, 118, 812–817.

Fontes, L.C.B., Sarmento, S.B.S., Spoto, M.H.F. & Dias, C.T.S. (2008). Preservation of minimally processed apple using edible coatings. *Food Science and Technology (Campinas)*, 28: 872-880.

Food and Agriculture Organization- FAO. (2013). *Food wastage foodprint, impacts on natural resources.* http://www.fao.org/docrep/018/i3347e/i3347e.pdf. Accessed 21 May 2018.

Fujii, H., Iwase, M., Ohkuma, T., Ogata-Kaizu, S., Ide, H., Kikuchi, Y., Idewaki, Y., Joudai, T., Hirakawa, Y., Uchida, K., Sasaki, S., Nakamura, U. & Kitazono, T. (2013). Impact of dietary fiber intake on glycemic control, cardiovascular risk factors and chronic kidney disease in Japanese patients with type 2 diabetes mellitus: the Fukuoka Diabetes Registry. *Nutrition Journal*, 12, 1-8.

Giordano, D., Locatelli, M., Travaglia, F., Bordiga, M., Reyneri, A., Coisson, J.D. & Blandino, M. (2017). Bioactive compound and antioxidant activity distribution in roller-milled and pearled fractions of conventional and pigmented wheat varieties*. Food Chemistry*, 233,483-491.

Gómez, M. & Martinez, M.M. (2017). Fruit and vegetable by-products as novel ingredients to improve the nutritional quality of baked goods. *Critical Reviews in Food Science and Nutrition*, 2017, 1-17.

Gondim, J.A.M., Moura, M.F.V., Dantas, A.S., Medeiros, R.L.S. & Santos, K.M. (2005). Centesimal composition and minerals in peels of fruits. *Food Science and Technology (Campinas)*, 25, 825-827.

Gotti, R., Amadesi, E., Fiori, J., Bosi, S., Bregola, V., Marotti, I. & Dinelli, G. (2018). Differentiation of modern and ancient varieties of common wheat by quantitative capillary electrophoretic profile of phenolic acids. *Journal of Cromatograpy A*, 1532, 208-215.

He, L., Xu, H., Liu, X., He, W., Yuan, F., Hou, Z. & Gao, Y. (2011). Identification of phenolic compounds from pomegranate (*Punica granatum* L.) seed residues and investigation into their antioxidant capacities by HPLC-ABTS+ assay. *Food Research International*, 44, 1161-1167.

Hidalgo, A., Ferraretto, A., De Noni, I., Bottani, M., Cattaneo, S., Galli, S. & Brandolini, A. (2018). Bioactive compounds and antioxidant properties of pseudocereals-enriched water biscuits and their *in vitro* digestates. *Food Chemistry,* 240, 799-807.

Infante, J., Selani, M.M., Toledo, N.M.V., Silveira-Diniz, M.F., Alencar, S.M. & Spoto, M.H.F. (2013). Atividade antioxidante de resíduos agroindustriais de frutas tropicais. *Alimentos e Nutrição*, 24, 87-91.

Leonel, S., Leonel, M. & Sampaio, A.C. (2014). Processamento de frutos de abacaxizeiro cv smooth cayenne: perfil de açúcares e ácidos dos sucos e composição nutricional da farinha de cascas. *Revista Braileira de Fruticultura*, 36, 433-439.

Mallek-Ayadi, S., Bahloul, N. & Kechaou, N. (2017). Characterization, phenolic compounds and functional properties of Cucumis melo L. peels. *Food Chemistry*, 15,1691-1697.

Martins, Z.E., Pinho, O. & Ferreira, I.M.P.L.V.O. (2017). Food industry by-products used as functional ingredients of bakery products. *Trends in Food Science and Technology*, 67, 106-128.

Mariod, A. & Matthaus, B. (2008). Fatty acids, tocopherols, sterols, phenolic profiles and oxidative stability of *Cucumis melo* var. agrestis oil. *Journal of Food Lipids*, 15, 56-67.

Miteva, D., Nacheva, I., Djakova, A. & Tsvetkov, T.S.V. (2008). Extended safe preservation period of foods of plant origin through combined technological methods. *Bulgarian Journal of Agricultural Science,* 14, 43-47.

Morais, D.R., Rotta, E.M., Sargi, S.C., Bonafe, E.G., Suzuki, R.M., Souza, N.E., Matsushita, M. & Visentainer, J.V. (2017). Proximate composition, mineral contents and fatty acid composition of different parts and dried peels of tropical fruits cultivated in Brazil. *Journal of the Brazilian Chemical Society*, 28, 308-318.

Müller, L., Fröhlich, K. & Böhm, V. (2011). Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (aTEAC), DPPH assay and peroxyl radical scavenging assay. *Food Chemistry*, 129, 139-148.

Packer, V.G., Melo, P.S., Bergamaschi, K.B., Selani, M.M., Villanueva, N.D., Alencar, S.M. & Contreras-Castillo, C.J. (2015). Chemical characterization, antioxidant activity and application of beetroot and guava residue extracts on the preservation of cooked chicken meat. *Journal of Food Science and Technology*, 52, 7409-7416.

Pathak, D., Majumdar, J., Raychaudhuri, U. & Chakraborty, R. (2016). Characterization of physicochemical properties in whole wheat bread after incorporation of ripe mango peel. *Journal of Food Measurement and Characterization*, 10, 554-561.

Paraman, I., Sharif, M.K., Supriyadi, S. & Rizvi, S.S.H. (2015). Agro-food industry byproducts into value-added extruded foods. *Food and Bioproducts Processing*, 96, 78-85.

Pereira, M. A. B., Siebeneichler, S.C., Lorençoni, R., Adorian, G.C., Silva, J.C., Garcia, R.B.M., Pequeno, D.N.L., Souza, C.M. & Brito, R.F.F. (2009). Qualidade do fruto de abacaxi comercializado pela Cooperfruto: Miranorte-TO. *Revista Brasileira de Fruticultura*, 31, 1049-1053.

Ragaee, S., Seetharaman, K. & Abdel-Aal, E.S.M. (2014). The impact of milling and thermal processing on phenolic compounds in cereal grains. *Critical Reviews in Food Science and Nutrition*, 54, 837-849.

Ramos, S., Martín, M.A. & Goya, L. (2017). Effects of cocoa antioxidants in type 2 diabetes mellitus. *Antioxidants*, 6, 1-16.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26, 1231–1237.

Rolim, P.M., Fidelis, G.P., Padilha, C.E.A., Santos, E.S., Rocha, H.A.O. & Macedo, G.R. (2018). Phenolic profile and antioxidant activity from peels and seeds of melon (*Cucumis melo* L. var. reticulatus) and their antiproliferative effect in cancer cells. *Brazilian Journal of Medical and Biological Research*, 51, e6069.

Sato, M.F., Vieira, R.G., Zardo, D.M., Falcão, L.D., Nogueira, A. & Wosiacki, G. (2010). Apple pomace from eleven cultivars: an approach to identify sources of bioactive compounds. *Acta Scientiarum,* 32, 29-35.

Selani, M.M., Brazaca, S.G.C., Dias, C.T.S., Ratnayake, W.S., Flores, R.A. & Bianchini, A. (2014). Characterization and potential application of pineapple pomace in an extruded product for fibre enhancement. *Food Chemistry*, 163, 23-30.

Selani, M.M., Bianchini, A., Ratnayake, W.S., W.S., Flores, Massarioli, A.P., Alencar, S.M. & Canniatti-Brazaca, S.G. (2016). Physicochemical, Functional and Antioxidant Properties of tropical fruits co-products. *Plants Foods for Human Nutrition,* 71, 137-144.

Singleton, V. L., Orthofer, R. & Lamuela, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. *Methods of Enzymology*, 299, 152–178.

Sivamaruthi, B.S., Kesika, P. & Chaiyasut, C. (2018). A comprehensive review on anti-diabetic property of rice bran. Asian *Pacific Journal of Tropical Biomedicine*, 8, 79-84.

Soares, M., Welter, L., Gonzaga, L., Lima, A., Mancini-Filho, J. & Fett, R. (2008). Avaliação da atividade antioxidante e identificação dos ácidos fenólicos presentes no bagaço de maçã cv. Gala. *Food Science and Technology (Campinas)*, 28, 727-732.

Storck, C.R., Nunes, G.L., Oliveira, B.B. & Basso, C. (2013). Folhas, talos, cascas e sementes de vegetais: composição nutricional, aproveitamento na alimentação e análise sensorial de preparações. *Ciência Rural*, 43, 537-543.

Tamanna, N. & Mahmood, N. (2015). Food processing and Maillard reaction products: effects on human health and nutrition. *International Journal of Food Science*, 2015, 526762.

Tanska, M., Roszkowska, B., Sylwester, C., Borowska, E.J., Bojarska, J. & Dabrowska, A. (2016). Effect of fruit pomace addition on shortbread cookies to improve their physical and nutritional values. *Plant Foods for Human Nutrition*, 71, 307-313.

Toledo, N.M.V., Nunes, L.P., Silva, P.P.M., Spoto, M.H.F. & Canniatti-Brazaca, S.G.C. (2017). Influence of pineapple, apple and melon by-products on cookies: physicochemical and sensory aspects. *International Journal of Food Science and Technology*, 52, 1185-1192.

Zbikowska, A. & Kowalska, M. (2017). The use of apple fiber as a fat substitute in the manufacture of bakery products. *Journal of Food Science and Preservation*, 41, e13241.

Zeb, A. (2016). Phenolic profile and antioxidant activity of melon (*Cucumis Melo* L.) seeds from Pakistan. *Foods*, 5, 1-7.

Wen, L. & Wrolstad, R.E. (2002). Phenolic composition of authentic pineapple juice. *Food Chemistry and Toxicology*, 67, 155-161.

Willats, W.G.T., Knox, J. P. & Mikkelsen, J.D. (2006). Pectin: new insights into an old polymer are starting to gel. *Trends in Food Science & Technology*, 17, 97-104.

3. INFLUENCE OF PINEAPPLE, APPLE AND MELON BY-PRODUCTS ON COOKIES: PHYSICOCHEMICAL AND SENSORY ASPECTS

ABSTRACT

Physicochemical and sensory effects were investigated regarding the partial substitution of wheat flour in cookies with flours made of pineapple, apple and melon by-products, in the concentrations 5%, 10% and 15%. Cookies with 15% of melon flour were prominent in relation to the nutritional aspects, especially about the contents of fibers (4.67% to 6.46%) and ash (1.74% to 2.25%). The use of these by-products resulted in slightly darker cookies, with variations in diameter and in the expansion factor. There was a positive influence of the use of the pineapple by-product concerning consumers' preference. Additionally, the cookies with 15% of pineapple by-product presented the highest acceptance rate (97%) and buying intention (53%). Therefore, the use of fruit by-products in the development of cookies is a viable alternative which can be explored for nutritional, technological and sensory purposes by the food industry.

Keywords: Fruits; Biscuits; Dietary fiber; Sensory evaluation; Flour

3.1. INTRODUCTION

The production of fruits has been growing mainly because of the expansion of the processed food market, especially for pulps, nectars, jams, sweets and minimally processed products. The high demand for these products is related to the current lifestyle, which requires highly practical and convenient products. Nevertheless, concomitant to the search for processed foods, the production of residues discarded by the industries increased, which might result in environmental damages and rise in costs for the industries (Jayathilakan *et al.*, 2012).

Among the main by-products generated after fruit processing are peels, seeds and pomace. Nowadays, fruit by-products are directed mainly to animal feeding or the pharmaceutical industry. However, this material might present significant amounts of fibers, vitamins, minerals, phenolic compounds and antioxidants; hence, it is suggested to be included in human feeding (Sancho *et al.*, 2015).

In this way, by-products of pineapple, apple and melon could be used as an alternative source of nutrients in the development of new foods. Bakery products, such as cookies, are considered the best vehicles to increment the nutritional value, for being versatile and presenting a high popularity and acceptance (Kurek & Wyrwisz, 2015).

Studies have been exploring the development of baked products containing by-products to improve nutritionally trivial market products (Mildner-Skudlarz *et al.*, 2013, De Camargo *et al.*, 2014; Reis, Rai & Abu-Ghannam, 2014). Nonetheless, there are still scarce data in literature referring to the physicochemical and sensory effects of the addition of by-products from the central axis of the pineapple, apple's endocarp and melon peels in bakery products. Pineapple, apple and melon are widely consumed and processed especially for the production of juice and pulp; thus, it becomes relevant to investigate possible applications of their by-products.

Therefore, the purpose of the present work was to incorporate by-products of fruit processing in cookies formulations with partial substitution of the wheat flour by flours made of pineapple, apple and melon by-products.

3.2. MATERIAL AND METHODS

3.2.1. Raw materials

The by-products of pineapple (central axis), apple (endocarp) and melon (peels) were provided by the industry of minimally-processed foods Della Natural (Engenheiro Coelho, SP, Brazil, 22º29'18" S and 47º12'54" W). In the factory, the fruits were washed and sanitized with chlorine dioxide (1 mL/L). The by-products were packed in polyethylene bags, transported and stored at -18ºC for a maximum of 30 days.

To obtain the flour made of fruit by-products, the material was dehydrated in an E-C Modulyo lyophilizer (Apparatus Inc., New York, USA) (-40ºC, 0.998 mbar, 96 hours). The dehydrated by-products were then ground in a knife mill (Marconi, Piracicaba, Brazil), sifted through a 35-mesh sieve (Abronzinox, 0.425 mm) and stored in polyethylene bags, properly closed and frozen (-18ºC).

The other ingredients employed in the cookies formulations, such as: wheat flour type 1 with 10% of protein (Dona Benta), refined sugar (União), baking powder (Royal), eggs (Qualitá), salted butter (Scala) and pieces of semisweet chocolate bar with 40% of cocoa (Nestlé) were acquired in the local market of Piracicaba, SP, Brazil (22º 43' 31" S and 47º 38' 57" W).

3.2.2. Cookies preparation

Ten formulations of cookies were developed, one of the treatments being the control cookie (CCT), which was prepared with only wheat flour, whereas the others were supplemented with by-products of the fruits: pineapple (CPI), apple (CAP) and melon (CML) in the concentrations 5%, 10% and 15%.

Initially, 105 g of sugar (sifted) and 75 g of butter (refrigerated and with a firm consistency) were mixed in a planetary-type mixer (Philips Walita, RI7915, China) for 10 seconds. Then, 50 g of egg were added and the dough was beaten for another 5 seconds.

A total of 200 g of flour was used to prepare the dough. According to the percentage of by-product desired in the cookies (0%, 5%, 10% or 15%), the amount in grams of wheat flour and by-product flour were calculated. The flours, together with 10 g of baking powder and 25 g of small pieces of semisweet chocolate were integrated into the cookie dough and the mixture was beaten for approximately 10 seconds or until the complete disappearance of the butter lumps.

The dough was then opened on transparent plastic packages with the aid of a roll and cut using circular stainless-steel molds. The cookies were baked at 180ºC for 10 min in an electric oven (Perfecta, Ponta Grossa, Brazil). Shortly after, they were cooled at room temperature and stored in glass containers.

3.2.3. Proximate composition

Ash, protein and lipids were determined according to AOAC (2010). For the determination of fibers, the method described by Asp, Johansson and Hallmer (1983) was employed. Carbohydrate content was determined by difference. The analyses were performed in quadruplicates.

3.2.4. Physicochemical analyses

Color analysis was conducted using the colorimeter Chroma Meter CR-400, 8mm diameter and standard illuminant C, Konica Minolta Sensing (Tokyo, Japan) to obtain the values of lightness(L), hue angle (h) and chroma (C) of the cookies surface. For pH determination, the samples were ground and diluted in water in the proportion 10% (w/v), and pH was measured in a potentiometer (Quimis, Q799-D2, São Paulo, Brazil). Water activity (a_w) was determined using an Aqualab instrument (Aqualab CX 2T Decagon Devices Inc., Pullman, USA). The analyses were performed in quadruplicate.

3.2.5. Post-baking properties

Weight variation in the cookies was evaluated by gravimetry (Marte UX-4200H, Minas Gerais, Brazil), based on the weight of the cookies before and 1 hour after baking, with the result expressed in percentage (%). The variation in cookies diameter was evaluated using a digital caliper, before and 1 hour after baking, and the result was expressed in percentage (%). Cookies thickness after baking was obtained by direct measurement in a digital caliper, with the result expressed in mm (millimeters). Cookies expansion factor was determined based on the ratio between diameter and thickness of the cookies after 1 hour of baking. All analyses were conducted using ten cookies samples for each treatment.

3.2.6. Microbiological aspects

Microbiological analyses were performed for *Staphylococcus aureus*, *Salmonella* spp. and coliforms at 45ºC before sensory evaluation (Downes & Ito, 2001).

3.2.7. Sensory analysis

The present study was approved by the Committee on Ethics in Research of the “Luiz de Queiroz” College of Agriculture, University of São Paulo (Piracicaba, Brazil), under the protocol number CAAE: 43425215.4.0000.5395. The cookies were prepared 24 hours before the tests, stored in polyethylene bags and maintained in a desiccator until the moment of the sessions.

Two methods were chosen for the sensory evaluation of the cookies: preference ranking test and acceptance test. In both, attention was given to the fact that the panelists did not present a history of allergy to the ingredients involved in the cookies formulations. Panelists were not trained before any tests.

3.2.7.1. Preference ranking test

The preference ranking test (ISO, 2006) was performed to reduce the number of initial treatments (*n*=10) evaluated by the panelists in the acceptance test, avoiding sensory fatigue. Thus, the treatments of higher preference among the panelists were selected for their future evaluation of acceptance.

For this test, the sensory team was composed of fifty-six panelists, workers and students of the “Luiz de Queiroz” College of Agriculture, with age between 18 and 50 years, 77 % of which were females.

The test was conducted in three sessions, one for each type of cookie (CPI, CAP and CML). In each session, the panelists received four units of cookies (6g), referring to the by-product percentage in the formulation (0%, 5%, 10% and 15%), previously coded with random 3-digit numbers and arranged in rectangular trays. The panelists tried the samples from the left to the right, drank water between them and placed them in increasing order of preference about their global aspect.

After performing the preference ranking test, for each treatment of cookie with fruit by-product, those presenting not only the highest preference among the panelists, but also the highest fiber concentration was selected for use in the acceptance test.

3.2.7.2. Acceptance test

In the acceptance test, the panelist evaluated four samples of cookies, one of them the control (CCT) and the others containing 15% of each fruit by-product (CPI 15%, CAP 15% and CML 15%), as these were the samples previously selected by the preference ranking test.

Sixty-two voluntary panelists participated in this test, workers and students of the “Luiz de Queiroz” College of Agriculture, with age between 18 and 50 years. Of these panelists, 65% were female and 74% declared to consume cookies at least once a week.

The panelists evaluated the samples using the nine-point hedonic scale (Moraes, 1993), which varies gradually from 1 (extremely disliked) to 9 (extremely liked). Each panelist evaluated four different cookies units (6g), coded with random 3-digit numbers and arranged in rectangular trays. The panelists tried the samples from the left to the right, drank water between them and used the hedonic scale to evaluate the attributes appearance, aroma, flavor, texture and global impression.

Cookies buying intention was assessed by the identification and the justification of the panelists regarding the samples they would buy. The acceptance rate (AR) was determined for each treatment, accounting the scores between 6 (slightly liked) and 9 (liked very much) obtained in the acceptance test for each cookie formulation, and comparing them to the total number of panelists participating in the test (Equation 1):

$$AR\ (\%) = \frac{\text{(scores between 6 and 9 obtained in the acceptance test) x 100}}{\text{Total of panelists in the acceptance test}} \quad (1)$$

3.2.8. Statistical analyses

The experimental design adopted in the physicochemical analyses was the completely randomized. Conversely, in the sensory preference ranking test, the Latin square design (4x4) was employed, and in the sensory acceptance test, the randomized complete block design was applied. The program Statistical Analysis System (SAS) model 9.2 was used. The data referring to the physicochemical analyses and sensory acceptance test were subjected to the analysis of variance (ANOVA) for test F and to the comparison of means by the Tukey's test (p<0.05). For the sensory preference ranking test, the difference between treatments was verified by the Table of Newell and MacFarlane (p<0.05).

3.3. RESULTS AND DISCUSSION

3.3.1. Proximate composition

Preliminary tests were performed with cookies that presented 17%, 18% and 20% of by-products. These cookies presented undesirable characteristics regarding raw dough malleability: those made with melon or apple flour presented a dry, crumbly and heterogeneous dough, while the dough in which pineapple flour was used had a low consistency and was sticky. In these cases, the standardization of cookies molding was a parameter impaired by the lack of dough consistency.

Even though food formulation with fruit by-products is desirable, care should be taken regarding technological problems: mixing properties of the dough (stability, mixing tolerance) and final product characteristics (volume, color, moisture and hardness) can be affected (Sudha, Vetrimani & Leelavathi, 2007). Furthermore, high levels of fruit flour were not interesting in sensory terms for denoting a strong residual flavor (usually a bitter taste). So, for this study the use of fruit flour concentrations above 15% in the formulations of cookies was considered unfeasible for the technological and sensory aspects.

The ash (Table 1) content is related to the minerals present in the food composition. In general, it was observed that the addition of fruit flours to the cookies significantly increased ($p<0.05$) the ash content, especially in treatments CML, with a significant difference ($p<0.05$) between CML 5%, CML10% and CML 15%. Ash content above those present in conventional biscuits are observed when fruit by-products are employed in the preparation of cookies (Uchoa *et al.*, 2009, Becker *et al.*, 2014; Naknaen *et al.*, 2016).

Table 1. Proximate composition of cookies made with 0% (CCT), 5%, 10%, and 15% of pineapple (CPI), apple (CAP) and melon (CML) by-products

Treatments	Ash	Protein	Lipid	Carbohydrate	Insoluble Fiber	Soluble Fiber
CCT	1.53±0.03[1]^{f}[2]	8.54±0.03^{d}	19.55±0.38^{a}	67.73	1.45±0.15^{ef}	1.20±0.13^{fg}
CPI 5%	1.64±0.02^{de}	8.14±0.02^{f}	18.37±0.20^{ef}	69.41	1.30±0.07^{f}	1.14±0.12f^{g}
CPI 10%	1.53±0.02^{f}	8.13±0.00^{f}	19.17±0.23^{bc}	68.32	1.68±0.12^{ef}	1.17±0.01^{fg}
CPI 15%	1.62±0.05^{e}	8.13±0.01^{f}	17.88±0.14^{f}	69.24	2.08±0.06^{d}	1.05±0.02^{g}
CAP 5%	1.66±0.05^{de}	8.87±0.05^{b}	18.17±0.24^{f}	68.46	1.23±0.13^{f}	1.61±0.25^{cde}
CAP 10%	1.66±0.02^{de}	8.51±0.02^{d}	18.87±0.12^{d}	67.55	1.96±0.21^{de}	1.45±0.10^{e}
CAP 15%	1.70±0.03^{cd}	8.37±0.00^{e}	19.06±0.97^{ab}	66.71	2.52±0.01^{c}	1.64±0.01^{d}
CML 5%	1.74±0.03^{c}	8.64±0.00^{c}	19.28±0.21^{abc}	65.67	2.90±0.14^{b}	1.77±0.08^{c}
CML 10%	1.94±0.04^{b}	8.82±0.02^{b}	18.20±0.11^{ef}	66.00	2.73±0.08^{b}	2.31±0.23^{b}
CML 15%	2.25±0.08^{a}	8.94±0.02^{a}	18.81±0.15^{d}	63.54	3.54±0.19^{a}	2.92±0.14^{a}

[1]Mean±standard deviation (*n=4*). [2]Means with different letters within a column indicate significant differences ($p<0.05$). Results are expressed as g/100g of dry basis.

Among the macronutrients, carbohydrates comprised the largest amount. This is justified because the major ingredient in cookie formulation is wheat flour, which presents 76% of carbohydrates (USDA, 2013). Carbohydrates values ranged between 63.54 (CML 15%) to 69.41% (CPI 5%). This fact was already expected, since the pineapple varieties usually present a carbohydrate amount of approximately 13%, whereas in melons these values are lower, varying from 6.5% to 9% (USDA, 2013).

Lipids, compounds which act as the main energy source to the organism, were the second class of macronutrients with great representativity. Treatments CPI 15% and CAP 5% presented the lowest lipid values. According to Jacob and Leelavathi (2007), the lipids are considered essential ingredients in cookie formulation, being present in concentrations between 30 and 60%, values which are considerably above those observed in this study. Butter can be considered the major responsible for the elevated lipid content in cookies, once it is composed of, in average, 81% of it (USDA, 2013).

The proteins were present in amounts of 8.13 to 8.94%, with the lowest value verified for treatments CPI. The values presented in this study were superior to those observed by Naknaen *et al.* (2016), which register variations between 4.84% and 5.54% for cookies with melon peels, but below the described by Uchoa *et al.* (2009), whose variations were between 10% and 27% for cookies prepared with cashew residues.

Regarding the fibers, treatments CML presented a significant increase in fiber content when compared to CCT. Higher values of insoluble than soluble fibers were observed in the cookies. It was observed that 100g of CML 15% presented 17% (man) and 26% (woman) of the Dietary Reference Intake (DRI) of fibers for an adult (Institute of Medicine, 2005), values considered expressive. It is known that the consumption of fibers is related to the reduction of the risk and the treatment of chronic diseases, such as obesity, cardiovascular diseases and diabetes (Fujii *et al.*, 2013).

3.3.2. Physicochemical analyses

Considering aw, it is possible to affirm that the addition of the fruit flours significantly increased (p<0.05) the water activity of cookies, except in treatment CPI 5%, which might have happened because of the rise in fiber contents and, consequently, of water retention capacity. Nevertheless, the results presented aw ≤ 0.42, which is satisfactory to increase products shelf life, besides avoiding loss in crunchiness and the development of microorganisms. The results obtained were below the reported by Naknaen *et al.* (2016), whose values were between 0.45 and 0.54.

Table 2. Parameters of pH, water activity (a_w), and color (L, C e h) of cookies made with 0% (CCT), 5%, 10%, and 15% of pineapple (CPI), apple (CAP) and melon (CML) by-products

Treatments	pH	a_w	Color		
			L	C	h
CCT	7.52±0.01[1a2]	0.34±0.00[d]	64.1±0.91[a]	36.26±0.58[ab]	76.49±0.61[a]
CPI 5%	7.04±0.02[d]	0.32±0.00[e]	57.76±1.11[bc]	36.38±0.84[ab]	70.99±1.64[b]
CPI 10%	6.92±0.03[e]	0.34±0.00[d]	56.33±1.53[bc]	36.23±0.88[ab]	70.53±0.43[b]
CPI 15%	6.91±0.03[e]	0.38±0.00[b]	52.45±1.73[d]	35.46±0.72[abc]	67.88±2.86[b]
CAP 5%	7.12±0.01[c]	0.36±0.00[c]	52.35±1.84[d]	34.52±0.30[bc]	68.14±1.81[b]
CAP 10%	7.00±0.02[d]	0.39±0.00[b]	54.77±1.48[bcd]	34.85±0.85[abc]	69.61±1.59[b]
CAP 15%	6.82±0.02[f]	0.41±0.00[a]	51.72±1.06[d]	33.7±0.74[c]	67.65±0.45[b]
CML 5%	7.23±0.03[b]	0.39±0.01[b]	58.21±2.31[b]	36.52±1.34[a]	70.19±1.32[b]
CML 10%	7.09±0.01[c]	0.42±0.01[a]	54.41±1.83[cd]	35.02±0.62[abc]	67.59±1.41[b]
CML 15%	7.04±0.02[d]	0.41±0.01[a]	58.42±0.80[b]	36.52±0.43[a]	69.88±0.84[b]

[1]Mean±standard deviation ($n=4$). [2]Means with different letters within a column indicate significant differences (p<0.05). Results are expressed on a dry weight basis.

Cookies color is usually formed during the baking process because of Maillard reaction, which occurs between reducing sugars and proteins. About color parameters, for lightness it was observed that treatment CCT presented a significantly higher value (p<0.05) than the others. In general, the use of fruit by-product flour in cookies formulation resulted in a decrease in L-values, effect also observed by Naknaen *et al.* (2016).

Concerning the parameter chroma, CAP 15% demonstrated a significantly lower value ($p<0.05$) than the other treatments, with a dull appearance. Observing hue angle (h), it is possible to affirm that CCT was the only treatment which differed significantly from the others ($p<0.05$), with the highest value for this parameter. The hue values varied between 67.59 and 76.49, which means the samples are closer to the yellow tone.

Other studies have also reported color alterations in cookies when wheat flour was partially substituted by fruit by-product flours (Becker *et al.*, 2014; Bhat & Ahsan, 2015; Naknaen *et al.*, 2016).

In relation to pH (Table 2), the highest value was observed for treatment CCT and the lowest for CAP 15%. The addition of fruit flours, in general, significantly reduced ($p<0.05$) the pH value of the samples. The results of this study are in accordance with those obtained by Uchoa *et al.* (2009), who registered values between 6.5 and 7.1 for cookies prepared with residues of guava and cashew.

The pH values of the cookies influence chemical reactions, such as Maillard reaction, generating alterations in color, flavor and texture of the product, parameters related to the perception and sensory acceptance of the consumers (Andrés-Bello *et al*., 2013). According Pearson's correlation coefficient, a correlation between the pH and color was observed for the parameters of lightness (r = 0.800) and also hue angle (r = 0.781). In this way, it can be affirmed that the higher were the pH values, the closer to white were the lightness values and closer to yellow were the hue angle values.

3.3.3. Post-baking properties

Weight variation in cookies provides data on weight loss during baking and, therefore, is related to the yield of cookies. Formulation CAP 15% presented the lowest weight variation and, thus, the highest yield. Among the other treatments, significant differences ($p>0.05$) were not observed regarding weight variation between raw and post-baking dough (Table 3).

Table 3. Weight variation, diameter variation, thickness, and expansion factor of cookies made with 0% (CCT), 5%, 10%, and 15% of pineapple (CPI), apple (CAP) and melon (CML) by-products

Treatments	% Weight variation	% Diameter variation	Thickness	Expansion factor
CCT	13.93±0.51[1a2]	17.08±3.63[df]	8.48±0.54[c]	5.13±0.25[e]
CPI 5%	14.78±1.05[a]	24.33±3.97[abc]	6.93±0.31[d]	6.89±0.36[b]
CPI 10%	14.12±0.73[a]	29.28±4.29[a]	7.21±0.31[d]	6.86±0.40[b]
CPI 15%	14.03±0.63[a]	25.84±3.74[ab]	6.69±0.49[d]	7.41±0.52[a]
CAP 5%	12.75±0.68[ab]	21.74±2.01[bc]	8.02±0.29[c]	5.75±0.22[c]
CAP 10%	13.68±1.02[ab]	20.25±3.45[cd]	7.89±0.33[c]	5.66±0.35[cd]
CAP 15%	11.62±3.26[b]	17.95±4.08[df]	8.54±0.38[bc]	5.27±0.22[de]
CML 5%	13.38±0.51[ab]	13.22±3.78[ef]	9.14±0.39[ab]	4.63±0.19[f]
CML 10%	13.44±0.70[a]	11.54±3.00[e]	9.33±0.83[a]	4.48±0.37[f]
CML 15%	13.22±0.40[ab]	11.94±2.39[e]	9.64±0.32[a]	4.26±0.18[f]

[1]Mean±standard deviation (*n*=10). [2]Means with different letters within a column indicate significant differences (p<0.05). Results are expressed on a dry weight basis.

In terms of cookies diameter variation, it was observed that this parameter increased after baking. Treatments CML presented the lowest values of diameter variation, with CML 10% and CML 15% being the only treatments which presented diameter variations inferior to CCT. On the other hand, the highest diameter variations were registered for the treatments in which pineapple flour was employed (CPI). Presumably, this fact occurred due to the action of bromelain, a protease present in pineapple, usually employed in bakery products, acting on the peptide bonds of gluten (Bala *et al.*, 2012). Cookies diameter corresponds to a quality indicator, once cookies with a larger diameter are considered more desirable (Bhat & Ahsan, 2015).

For cookies thickness, all treatments where apple flour was employed (CAP) did not differ from CCT. A similar result was reported by Bhat and Ahsan (2015), who did not observe differences in the thickness of cookies prepared with more than 5% of tomato pomace powder in relation to the control. It is still worth highlighting that, for the cookies prepared with the same by-product, no significant differences (p>0.05) were observed among the concentrations employed.

For the expansion factor (EF), the highest values were observed in cookies CPI, especially CPI 15%. Comparatively, cookies CML presented the lowest EF values, without significant difference (p>0.05) among the different by-product concentrations employed. For cookies CAP, EF was inversely proportional to the addition of fruit by-product flour. The results are consistent with the reported by Becker *et al.* (2014) and Naknaen *et al.* (2016) in the sense that by-product incorporation to the cookies reduced EF values due to the water absorption capacity of the fibers present in the fruit by-products.

3.3.4. Sensory analysis

Microbiological analyses have been conducted before the sensory analyses and all cookie treatments were considered suitable for consumption.

In the preference ranking test (Table 4), it can be affirmed that there were no significant differences (p>0.05) between the cookies with fruit by-product and the CCT. Once the ranking test does not explain why a certain treatment is preferred over the others, an acceptance test was performed to verify which attribute is higher or lower in each cookie formulation. Thus, as there was no difference between the treatments, those with the highest fiber concentration (CPI 15%, CAP 15%, and CML 15%) were selected to be evaluated in the acceptance test.

Table 4. Preference ranking test (n=56) of cookies made with 0% (CCT), 5%, 10%, and 15% of pineapple (CPI), apple (CAP) and melon (CML) by-products

Treatments	1	2	3	4	5	6	7	8	9	10
Rank sums	70[a1]	91[a]	99[a]	100[a]	97[a]	91[a]	78[a]	85[a]	98[a]	80[a]
					Difference values					
1 (CCT)		21	29	30	27	21	08	15	28	10
2 (CPI 5%)			08	09	06	0	13	06	07	11
3 (CPI 10%)				01	02	08	21	14	01	19
4 (CPI 15%)					03	09	22	15	02	10
5 (CAP 5%)						06	19	12	01	17
6 (CAP 10%)							13	06	07	11
7 (CAP 15%)								07	20	02
8 (CML 5%)									13	05
9 (CML 10%)										18
10 (CML 15%)										

[1]Different letters within a row indicate significant differences (p<0.05).

Observing the sensory acceptance of the cookies (Fig.1A), it is possible to affirm that the addition of fruit flour did not influence attributes such as appearance and aroma of the samples. On the other hand, attributes such as flavor, texture and global impression varied. Treatment CPI 15% had a significantly (p<0.05) superior score (7.7) to the control treatment (CCT), which, in the hedonic scale, stayed close to the classification “liked very much”.

Comparatively, CML 15% was the cookie with the lowest scores for the parameters texture (6.8), flavor (5.9) and global impression (6.3), with values close to the classifications “slightly liked” and “moderately liked”. These low values attributed to the melon cookies can be related to the slight bitterness and acidity present in the by-products of the peels of this fruit, which occur because of the high polyphenol content (Naknaen *et al.*, 2016). In spite of presenting a lower score regarding global impression compared to the other treatments, CML 15% reached a similar mean to the other formulations observed in literature, which have also been evaluated by the 9-point hedonic scale (Uchoa *et al.*, 2009; Becker *et al.*, 2014 Naknaen *et al.*, 2016).

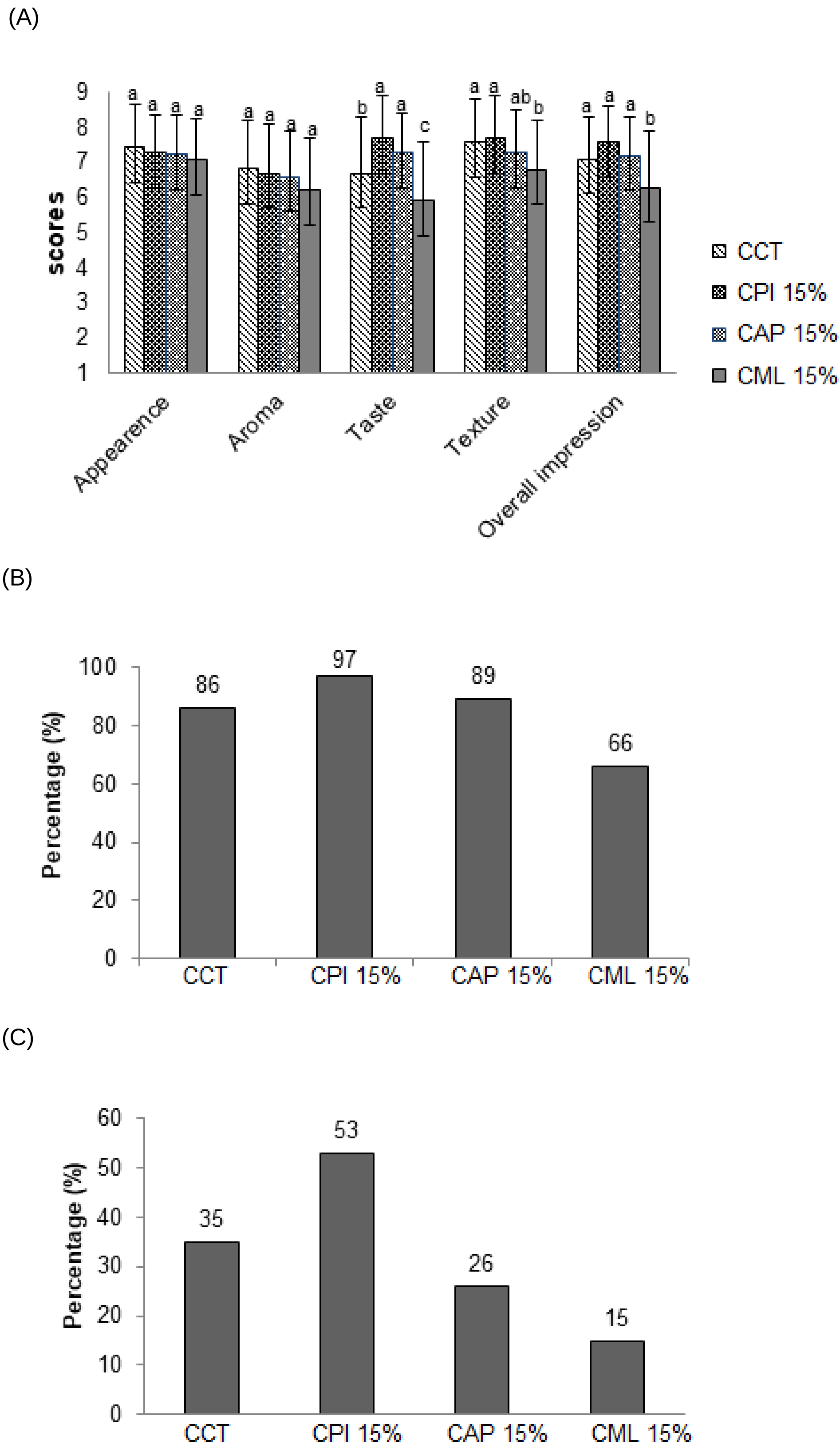

Figure 1. Acceptance test (A), acceptance rate (B), and buying intention (C) (*n*=62) of cookies control (CCT) and cookies made with 15% of pineapple (CPI 15%), apple (CAP 15%) and melon (CML 15%) by-products.

Evaluating the cookies acceptance rate (Fig. 1B), treatment CPI 15% was verified to reach the highest acceptance percentage (97%), followed by CAP 15%, CCT and CML 15%. It is worth highlighting that all treatments had an acceptance superior to 65%, which denotes an expressive acceptance of the panelists for the consumption of cookies prepared with fruit by-products.

From cookies buying intention (Fig. 1C), it can be affirmed that all panelists declared they would buy at least one of the cookies prepared with the fruit by-products. In accordance with the acceptance rate, buying intention reinforced the prevalence of treatment CPI 15%. Among the factors mentioned by the panelists for the choice of buying CPI 15% are a pleasant taste, crunchy texture, attractive appearance and the fact that this is an innovative product in the market, for both its healthiness appeal (related to the presence of fibers), and the sustainability aspect associated to the use of fruit by-products.

3.4. CONCLUSIONS

The use of fruit by-products positively influenced nutritional aspects of the cookies, especially regarding their fiber content, with the most expressive results (4.67% to 6.46%) obtained for the treatments using the flour composed of melon peels. It was verified that the high by-product concentration resulted in darker cookies, besides promoting variations in their diameter and expansion factor. Cookies prepared with flour made of pineapple central axis were more interesting in the sensory point of view, with CPI 15% presenting the highest acceptance and buying intention. Therefore, we conclude that the partial substitution of wheat flour by fruit by-product powder is a viable alternative, attributing nutritional, technological or sensory advantages to the cookies, depending on the by-product employed.

REFERENCES

Andrés-Bello, A., Barreto-Palancios, V., García-Segovia, P., Mir-Bel, J. & Martínez-Monzó, J. (2013). Effect of pH on color and texture of food products. *Food Engineering Reviews*, 5, 158-170.

AOAC Association of Official Analytical Chemists. (2010). Official methods of analysis of the Association of Official Analytical Chemists. 18th ed. Gaithersburg, USA: AOAC.

Asp, N. G., Johansson, C. G. & Hallmer, H. (1983). Rapid enzimatic assay of insoluble and soluble dietary fiber. *Journal of Agricultural and Food Chemistry*, 31, 476-482.

Bala, M., Ismail, N.A., Mel, M., Jami, M.S., Salleh, H.M. & Amid, A. (2012). Bromelain
production current trends and perspective. *Archives des Sciences*, 65, 369-399.

Bhat, M.A. & Ahsan, H. (2015). Physico-chemical characteristics of cookies prepared with tomato pomace powder. *Food Processing & Technology*, 7, 2-4.

Becker, F.S., Damiani, C., De Melo, A.A.M., Borges, P.R.S. & Boas, E.V.B.V. (2014). Incorporation of buriti endocarp flour in gluten-free whole cookies as potencial source of dietary fiber. *Plants Foods for Human Nutrition,* 69, 344-530.

De Camargo, A.C., Vidal, C.M.M., Canniatti-Brazaca, S.G. & Shahidi, F. (2014). Fortification of cookies with peanut skins: effect on the composition, polyphenols, antioxidant properties, and sensory quality. *Journal of Agricultural and Food Chemistry*, 62, 11228-11235.

Downes, F.P. & Ito, K. (2001). Compendium of methods for the microbiological examination of food. 4th ed. Washington, DC: American Public Health Association.

Fujii, H., Iwase, M., Ohkuma, T., Ogata-Kaizu, S., Ide, H., Kikuchi, Y., Idewaki, Y., Joudai, T., Hirakawa, Y., Uchida, K., Sasaki, S., Nakamura, U. & Kitazono, T. (2013). Impact of dietary fiber intake on glycemic control, cardiovascular risk factors and chronic kidney disease in Japanese patients with type 2 diabetes mellitus: the Fukuoka Diabetes Registry. *Nutrition Journal*, 12, 1-8.

Institute of Medicine. (2005). *Dietary references intakes: energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids*. Washington, DC: National Academies Press, 339-421.

ISO International Organization for Standardization. (2006). ISO 8587:2006. Sensory Analysis – Methodology – Ranking. Geneva, Switzerland: ISO.

Jacob, J. & Leelavathi, K. (2007). Effect of fat-type on cookie dough and cookie quality. *Journal of Food Engineering*, 79, 299-305.

Jayathilakan, K., Sultana, K., Radhakrishna, K. & Bawa, A.S. (2012). Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review. *Journal of Food Science and Technology*, 49, 278-293.

Kurek, M. & Wyrwisz, J. (2015). The application of dietary fiber in bread products. *Journal of Food Processing & Technology*, 6, 1-4.

Mildner-Szkudlarz, S., Bajerska, J., Zawirska-Wojtasiak, R. & Górecka, D. (2013). White grape pomace as a source of dietary fibre and polyphenols and its effect on physical and nutraceutical characteristics of wheat biscuits. *Journal of the Science of Food and Agriculture*, 93, 389-395.

Moraes, M. A. C. (1993). Métodos para avaliação sensorial dos alimentos. 8th ed. Campinas: UNICAMP, 93 p.

Naknaen, P., Itthisoponkul, T., Sondee, A. & Angsombat, N. (2016). Utilization of watermelon rind waste as a potential source of dietary fiber to improve health promoting properties and reduce glycemic index for cookie making. *Food Science and Biotechnology*, 25, 415-424.

Reis, S.F., Rai, D.K. & Abu-Ghannam, N. (2014). Apple pomace as a potencial ingredient for the development of new functional foods. *International Journal of Food Science & Technology*, 49, 1743-1750.

Sancho, S.O., Silva, A.R.A., Dantas, A.N.S., Magalhães, T.A., Lopes, G.S., Rodrigues, S., Costa, M.C., Fernandes, F.A.N. & Silva, M.G.V. (2015). Characterization of the industrial residues of seven fruits and prospection of their potential application as food supplements. *Journal of Chemistry*, 2015, Article ID 264284.

Sudha, M. L., Vetrimani, R. & Leelavathi, K. (2007). Influence of fibre from different cereals on the rheological characteristics of wheat flour dough and on biscuit quality. *Food Chemistry*, 100, 1365-1370.

Uchoa, A.M.A., Correia da Costa, J.M., Maia, G.A., Meira, T.B., Sousa, P.H.M. & Brasil, I.M. (2009). Formulation and physicochemical and sensorial evaluation of biscuit-type cookies supplemented with fruit powders. *Plant Foods for Human Nutrition*, 64, 153-159.

USDA United States Department of Agriculture. (2013). *USDA Food Composition Databases*. http://ndb.nal.usda.gov/ndb/search/list. Accessed 10 October 2017.

4. CHANGES IN THE SENSORY PROFILE AND PHYSICOCHEMICAL ASPECTS OF COOKIES CAUSED BY THE REPLACEMENT OF WHEAT FLOUR BY APPLE, PINEAPPLE AND MELON BY-PRODUCTS

ABSTRACT

This study aimed to investigate the influence of the replacement of wheat flour by fruit by-products on the sensory profile and physicochemical aspects of cookies. The biscuits were elaborated with 15% of apple, pineapple, and melon by-products, besides to a control treatment. Cookies were evaluated using Quantitative Descriptive Analysis (QDA) by eleven trained panelists through a 10 cm unstructured scale. The attributes analyzed were brown color, characteristic appearance, regular size, characteristic aroma, sweet aroma, fruity aroma, sweet taste, bitter taste, fruity taste, residual taste, bite resistance, crunchiness, and fracturability. The determination of moisture content and hardness were also conducted. The results were submitted to multivariate analysis using correlation, principal component analysis and cluster analysis. The values for moisture (%) ranged from 3.57 to 5.71, whereas for hardness (N) the results were between 44.04 and 72.49. Regarding texture, pineapple cookies presented desirable characteristics as crunchiness, bite resistance and hardness. The melon by-product influenced on the sensory profile of cookies, developing aroma and flavor of fruit; on the other hand, undesirable characteristics such as fracturability, bitter taste and residual flavor were reported in this treatment. Cookies containing apple by-product presented high moisture (5.52%) and consequently developing unsatisfactory crunchiness and bite resistance. It was concluded that the application of fruit by-products on cookies affects their sensory profile. The formulation prepared with pineapple by-product was the treatment that demonstrated more desirable attributes for cookies.

Keywords: Biscuit; Moisture; Hardness; Descriptive quantitative analysis; Residue; Co-product; Waste

4.1. INTRODUCTION

Cookies are popular foods, ready for consumption and with low cost, which usually have great acceptance. Nevertheless, many of these products are still formulated exclusively with wheat flour or other refined flours, which offer low nutritional quality due to the low amount of nutrients and high energy content. Considering that cookies can act as carriers of nutrients, the use of the nutritional supplementation strategies becomes essential (Saponjac *et al.*, 2016).

Fruits such as apple, pineapple, and melon are popular in the market and are consumed both *in natura* and processed, highlighting juices, pulps, jellies and handmade sweets. Fruit processing is one of the agro-industrial activities that most generate by-products. It is estimated that 25 to 80% of by-products can be produced depending on the fruit to be processed (Van Dyk *et al.*, 2013). The by-products obtained commonly present high nutritional value and are rich in fiber, vitamins, minerals and antioxidant compounds.

Nowadays, there are still few applicable alternatives for most vegetable by-products, which are frequently used as fertilizer or animal feed (Sancho *et al.*, 2015). The adequate use of these by-products may minimize environmental damage, add value to products and reduce the costs of industrialization, contributing to a more integrated and sustainable production (Jayathilakan *et al.*, 2012). Thus, several studies have explored the application of by-products in foods (Sharma *et al.*, 2012, De Camargo *et al.*, 2004; Ferreira *et al.*, 2015), promoting the reduction of food by-product discards and creating new forms of integral use of foods.

The development of new food products, however, is a complex process that requires the study of different aspects, among which, it is possible to emphasize the sensory analysis that is commonly used to analyze and interpret reactions of the characteristics of foods and as they are perceived by the senses of sight, smell, flavor, touch, and hearing. The quantitative descriptive sensory analysis is the most sophisticated of the methodologies available to the sensory professional. Its provide a complete sensory description of the tested product, that is, the basis for mapping of the similarities and disparity of different samples. In addition, it allows relating a specific ingredient or variables of the production process to the sensory attributes (Stone & Sidel, 2004).

Therefore, the present study aimed to investigate the influence of the partial replacement of wheat flour by flour of different by-products (pineapple, apple, and melon) regarding the physicochemical characteristics (moisture and texture) and sensory profile of cookies. In this sense, it is expected to contribute to the incentive of the application of fruit by-products in the development of new food products.

4.2. MATERIAL AND METHODS

4.2.1. Raw materials

The fruit by-products: pineapple (central axis), apple (endocarp) and melon (peel) were donated by the minimally processed food industry Della Natural (Engenheiro Coelho, SP, Brazil). The material was dehydrated in E-C Modulyo lyophilizer (Apparatus Inc., New York, USA) for 96 h at -40°C and pressure of 0.998 mbar. The dehydrated by-products were milled in a knife mill (Marconi, Piracicaba, Brazil), sieved in 35-mesh sieve (Abronzinox, 0.425 mm) and stored in polyethylene bags, properly closed and frozen (-18ºC). The other ingredients used in the formulations of cookies, such as wheat flour (type 1, 10% protein, Dona Benta), refined sugar (União), baking powder (Royal), eggs, salted butter (Scala) and dark chocolate (40% cocoa, Nestlé) were acquired in the local market of Piracicaba, SP, Brazil.

4.2.2. Cookie preparation

For the cookie preparation, the methodology proposed by Toledo *et al.* (2017) was used. Initially, 105 g of sugar and 75 g of butter were homogenized in a planetary-type mixer (10 s), which was followed by the addition of 50 g of eggs and the mixture was stirred for 5 s. Then, 170 g of wheat flour and 30 g of by-product flour (15% replacement of wheat flour per by-products flour) were added in the dough. For the CCT (control), only wheat flour (200 g) was used. Finally, 10 g of baking powder and

25 g of chocolate were added and mixed for 10 s. The cookies were cut with a circular mold and baked in an electric oven (Perfecta, Ponta Grossa, Brazil) at 180°C for 10 minutes. The samples were stored in glass containers at room temperature (25ºC) until the physicochemical and sensory analyzes, in order to avoid changes in the moisture and texture. In total, four formulations were prepared: the control (CCT), and the cookies added with 15% of fruit by-products: pineapple (CPI), apple (CAP) and melon (CML). The formulations were performed in three independent batches.

4.2.3. Physicochemical analyses

The moisture was determined by gravimetry according to AOAC (2010) and the analysis was perfomed in triplicate. The hardness was measured using the TA-XT texturometer (Stable Micro Systems, Godalming, United Kingdom). The cookies were placed horizontally on a platform and a rectangular steel sheet with dimensions of 12x7 cm, speed of 5 mm/s and a distance of 7.5 mm was used to cut them in half. The analysis was performed with 15 cookies for each formulation, 24 h after their baking. The results were expressed in N (Newton).

4.2.4. Quantitative descriptive analysis (QDA)

The present study was approved by the Research Ethics Committee of the "Luiz de Queiroz" College of Agriculture, University of São Paulo (Piracicaba, Brazil), under the protocol number CAAE: 43425215.4.0000.5395. The QDA was performed according to Moskowitz (1983), Stone and Sidel (2004), Meilgaard, Civille and Carr (2006). The attributes of appearance, flavor, aroma, and texture were evaluated and the data were compiled by the Compusense Five software (2010).

Initially, twenty-one panelists were invited to participate in a pre-selection to assess the ability in discriminating basic tastes and then to investigate the capacity to detect sweet and bitter flavors through sequential triangular test analyses. Among the panelists, eighteen obtained 100% of correct answers in the test and were selected to define the descriptive terminology for the sensory attributes of cookies. In this step, the CCT and CPI treatments were employed and represented the negative control (cookie without by-product flour) and the positive control (cookie containing fruit by-product), respectively. In addition, the CPI was chosen since in previous acceptance test (Toledo *et al.*, 2017), it was the treatment with fruit by-product that presented the highest acceptability.

From a consensus among the team, the similar descriptive terms that best described the cookies samples were grouped: brown color (BC), characteristic appearance (CP), regular size (SZ) and homogeneous appearance (HA) as appearance attributes; characteristic aroma (CA), sweet aroma (SA) and fruity aroma (FA) as aroma attributes; sweet taste (ST), bitter taste (BT), fruity flavor (FF) and residual flavor (RF) as flavor attributes; and bite resistance (BR), crunchiness (CC) and fracturability (FR) as texture attributes. Discrepant terms were eliminated. Then, the reference material (Table 1) was established and used for training panelists regarding the use of the descriptive terms to describe and quantify the intensity of each attribute.

Table 1. Attributes, definitions and references materials of scale extremes used in QDA of cookies

APPEARANCE		
Attribute	**Definition**	**Reference material**
Brown color	Refers to the intensity of the brown color of cookies	●Little: cookie with less bake time (8 minutes) ●Very: burnt cookie
Regular size	Refers to the circular shape and regular thickness of cookies; absence of deformations.	●Little: hand-crafted cookie with deformations in its shape ●Very: cookie made with a circular mold
Characteristic	Refers to the proper appearance of cookies	●Little: honeycomb biscuit with chocolate coating (Panco) ●Very: vanilla mini cookie with chocolate drops (Taeq)
Homogeneous	Refers to the general unifomity of cookies; absence of dark spots; absence of chocolate drops; color uniformity; absence of grooves.	●Little: hand-crafted cookie with chocolate drops, sugar granules and fibers; irregular shape and thickness ●Very: honeycomb biscuit without chocolate coating (Panco)
AROMA		
Attribute	**Definition**	**Reference material**
Characteristic	Refers to the typical aroma of cookies	●Little: cookie added with vinegar Very: commercial cookies (Taeq)
Sweet	Refers to the sweet aroma of cookies	●Little: milk biscuit (Marilan) ●Very: honeycomb biscuit with chocolate coating (Panco)
Fuit	Refers to the fruity aroma of cookies	●None: milk biscuit (Marilan) ●Very: pineapple sandwich biscuit (Piraquê)
FLAVOR		
Attribute	**Definition**	**Reference material**
Sweet	Refers to the sweet taste of cookies	●Little: whole cookies (Vitao) ●Very: biscuit of guava wrapped in sugar (Disnei)
Fruit	Refers to the fruity flavor of cookies	● None: milk biscuit (Marilan) ●Very: pineapple sandwich cookies (Piraquê)
Bitter	Refers to the bitter taste of cookies	● None: milk biscuit (Marilan) ●Very: cereal biscuit (Magic toast, Marilan) added with coffee and no sugar
Residual	Refers to the residual taste, aftertaste of cookies. Taste that remains on the palate of after eating the cookies.	● None: milk biscuit (Marilan) ●Very: cereal biscuit (Magic toast, Marilan) added with coffee and no sugar
TEXTURE		
Attribute	**Definition**	**Reference material**
Bite resistance	Refers to the force to break the cookies with the incisor teeth and the sound produced.	● Little: honeycomb biscuit with chocolate coating (Panco) ●Very: whole cookies (Vitao)
Crunchiness	Refers to the intensity of noise (popping sound) produced during chewing of the cookies	● Little: honeycomb biscuit with chocolate coating (Panco) ● Very: whole cookies (Vitao)
Fracturability	Refers to the tendency of fracture, breakage or disintegration of cookies after application of small force or impact.	●Little: chocolate sandwich biscuit (Trakinas) ●Very: cereal biscuit (Magic toast, Marilan) added with coffee and no sugar

After six training sessions, eleven panelists were selected for the final tests according to their ability at discriminating attributes, the repeatability in the evaluation and consensus with other team members. The final team was composed by students and employees from the research center, aged between 17 and 35 years, composed by 55% women, which declared to consume cookies between 1 and 3 times a week. The final sensory evaluation was performed in duplicate (two sessions). The tests were conducted in the morning, in individual booths equipped with fluorescent lamps. The cookies (10 g) were placed in white ceramic plates coded with three-digit random numbers. The samples (CCT, CPI, CAP, and CML) were presented in a sequential monadic way. Panelists were asked to rinse their mouths between the samples and indicate, using a non-structure 10-cm long scale, the sensory intensities perceived for each attribute.

4.2.5. Statistical analyses

For the physicochemical analyses, a completely randomized design was used, whereas for the QDA, the randomized complete block design was employed. The physicochemical results were expressed as mean ± standard deviation. The statistical difference between the means was determined by Tukey's test (p<0.05). In the statistical analysis of the data related to the sensory analysis, which are represented by the variables BC, CP, SZ, HA, CA, SA, FA, ST, BT, FF, RF, BR, CC and FR (sensory attributes) and by the observations CCT, CPI, CAP and CML (different cookies formulations), a multivariate analysis technique was used to evaluate concurrently all the of the data set obtained. Thus, the data were submitted to Principal Components Analysis (PCA), which, based on the matrix of correlation, enabled the sensory characterization of the cookies. The correlation analysis (CORR) through the Pearson's coefficient was determined for the evaluation of the interdependence between the variables, and calculation of the correlation matrix. For CORR, values higher than │0.70│ were considered accented indexes (Sokal & Rohlf, 1980).

A cluster analysis was also performed as a multivariate statistical method of data analysis, allowing the division of the set of observations in a number of homogeneous groups, according to the criterion of homogeneity of average distance between clusters, with a cutoff at 0.75, based on the characteristics of each observation. The software used to perform the statistical analyses was the SAS 9.3 (Statistical Analysis System Institute, Cary, NC, USA 2010).

4.3. RESULTS AND DISCUSSION

4.3.1. Physicochemical analyses

Regarding the moisture (Table 2), the control treatment (CCT) presented lower values than those with fruit by-products in their formulation (except CPI). According to the literature, cookies elaborated only with wheat flour usually present moisture between 2.55 and 5.84% (Becker *et al.*, 2014; Naknaen *et al.*, 2016); whereas cookies containing 15% of buriti endocarp flour presented amounts of

7.07% (Becker *et al.*, 2014) and cookies with 2.5% peanut skin demonstrated results above 10% (De Camargo *et al.*, 2014). This increase in the moisture of the cookies is associated with the greater amount of fibers coming from the by-products present in their formulations. As demonstrated in a previous study (Toledo *et al.*, 2017), the CAP and CML cookies are richer in fiber when compared to CCT and CPI. Thus, the high hydrophilic capacity of some types of fibers is known, which may retain water more easily, modifying the final moisture of the cookies (Canalis *et al.*, 2017).

Table 2. Moisture content and hardness of the control (CCT) and cookies containing 15% of pineapple (CPI), apple (CAP) and melon (CML) by-products.

	Moisture (%)	**Hardness (N)**
CCT	4.73±0.10[1b2]	62.82±5.69[b]
CPI	3.57±0.20[c]	72.49±6.32[a]
CAP	5.52±0.29[a]	44.04±4.50[d]
CML	5.71±0.15[a]	54.48±5.03[c]

[1]Mean±standard deviation (n=3). [2]Means with different letters within a column indicate significant differences (p<0.05).

As for the texture, it was observed that the CPI presented the highest value for hardness, while lower values were recorded for CAP and CML. Naknaen *et al.* (2016) also reported lower hardness for cookies with fruit by-products (watermelon peel flour). On the other hand, Herken *et al.* (2017) did not find significant differences between the hardness of the control treatment and of the cookies with up to 4% addition of mahaleb seeds. Although the replacement of wheat flour by fruit by-products reduces the amount of available gluten in the dough, unlike other biscuits and baked goods, the development of gluten is not a necessary step in the manufacture of cookies, which are considered short dough biscuits, that is, their dough has little extensible feature (Canalis *et al.*, 2017). Therefore, other factors such as increased moisture, fiber amount, diameter oscillations, thickness and spread ratio of the cookies may be responsible for changes in the texture of cookies (Herken *et al.*, 2017).

4.3.2. Sensory aspects

4.3.2.1. Correlation analysis (CORR)

The influence of the addition of fruit by-products on cookies is important not only for the physicochemical aspect but also to evaluate changes in the sensory profile of products. Concerning the data obtained by the CORR analysis, nine correlations above $|0.90|$ were observed (Table 3), which were considered very strong.

Table 3. Strong correlations coefficients observed for the variables studied

Variables	Correlation coefficients
Brown color – Fruity aroma	0.9032
Brown color – Bitter taste	0.9362
Sweet aroma - Characteristic aroma	0.9717
Sweet aroma – Crunchiness	0.9303
Residual flavor- Fruity aroma	0.9319
Residual flavor – Fruity flavor	0.9136
Residual flavor – Sweet taste	-0.9896
Fruity flavor – Bitter taste	0.9862
Fruity flavor – Sweet taste	-0.9510

The brown color variable presented very strong positive correlations with the variables fruity aroma and bitter taste. The addition of the by-products in cookies is associated with the increase in the concentration of fibers and phenolic compounds (Sharma *et al.*, 2012; De Camargo *et al.*, 2014). Fibers can promote changes in the color of the products, making them darker and brownish (Becker *et al.*, 2014; Naknaen *et al.*, 2016); whereas phenolic compounds can generate bitterness and astringency (Sivam *et al.*, 2010). It is noteworthy the effects caused by the Maillard reaction in the color of the cookies after baking, especially to the formulations containing fruit by-products. This reaction may occur as the fruits are sources of reducing sugars (Siti Roha *et al.*, 2013) while eggs and wheat flour (present in the cookies formulation) act as sources of amino acids, providing changes in the final color of the biscuits.

As for the fruity aroma, the presence of fruit by-products also tends to highlight this characteristic in cookies due to the presence of volatile compounds such as esters and terpenes (Zheng *et al.*, 2012; Yang *et al.*, 2012). The variables fruity aroma and fruity flavor had a very strong positive correlation with residual flavor, suggesting that the greater the presence of fruit by-products, the greater will be the residual flavor found in the cookies, possibly due to the presence of phenolic compounds (e.g. catechins), which are associated with bitter aftertaste (Sivam *et al.*, 2010).

For the sweet aroma, a very strong positive correlation was observed with variables characteristic aroma and crunchiness. Sugar is one of the most widely used ingredients in cookies formulations, being sucrose the most industrially used sugar. Among its functions, it is possible to emphasize the influence on the flavor, aroma, size, color, and texture (Gallagher *et al.*, 2003). During the preparation of the cookie dough, the sucrose dissolves totally or partially and recrystallizes again after baked, affecting strongly the texture, and greater amounts of sucrose tend to present products of harder texture, dry and friable (Handa, Goomer & Siddhu, 2012). It is worth mentioning that some fruit by-products, such as the central axis of the pineapple, present significant amounts of sugars (2.24% fructose, 2.56% glucose and 8.92% sucrose) (Siti Roha *et al.*, 2013), which may influence in the crunchiness and sweet aroma of cookies. Regarding the aroma, Nishibori and Bernhard (1993) demonstrated that sugars such as fructose and glucose can react with beta-alanine in cookies, producing the 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (DDMP), which is considered the main component associated with the sweet aroma in cookies.

Otherwise, the variable sweet taste presented very strong negative correlation with the variables fruity flavor and residual flavor. These attributes are related to the use of fruit by-products in cookies. Considering that the same amount of refined sugar was added in all formulations of cookies, it can be said that the perception of sweet taste was more intense in the samples that did not contain fruit by-products. It is believed that sweetness can mask the perception of other flavors, and generally the glucose is the sugar most highly associated with sweetness perception (Chadwick *et al.*, 2016).

4.3.2.2. Principal component analysis (PCA) and cluster analysis

The principal component analysis (PCA) (Figure 1) is represented by the projection of the variables (Figure 1A) and the observations (Figure 1B). In this study, two PCs were extracted from the total data set (Table 4), explaining 87.11% of the variance. The first Principal Component (PC1) explained 57.14% of the statistical variance and was positively correlated with the variables brown color (BC), homogeneous appearance (HA), fruity aroma (FA), fruity flavor (FF), bitter taste (BT) and residual flavor (RF) and negatively with the sweet taste (ST) and bite resistance (BR) variables. The second Principal Component (PC2) explained 29.97% of the statistical variance and was positively correlated only with the variables of characteristic aroma (CA), sweet aroma (SA), fracturability (FR) and crunchiness (CC). Variables SZ and CP were not correlated in the PCs because they did not obtain relevance in the scientific basis of the results.

Table 4. Total dataset of quantitative descriptive analysis of cookies

	CCT	CPI	CAP	CML
Brown color	2.95	5.29	5.97	6.73
Regular size	7.29	8.29	8.03	7.94
Characteristic appearance	7.08	7.65	7.37	6.71
Homogeneous appearance	4.66	4.41	5.09	4.94
Characteristic aroma	6.54	7.43	5.61	6.00
Sweet aroma	4.26	4.76	3.16	3.79
Fruity aroma	0.51	2.03	1.66	3.33
Sweet taste	5.17	5.04	4.87	4.10
Fruity flavor	0.57	1.26	2.73	4.35
Bitter taste	0.54	1.11	1.95	2.52
Residual flavor	0.79	1.16	1.17	2.55
Bite resistance	5.75	5.42	4.11	4.46
Crunchiness	5.40	6.74	3.91	5.65
Fracturability	4.37	6.39	4.15	6.80

CCT: cookie control; CPI: cookie containing pineapple by-product; CAP: cookie containing apple by-product; CML: cookie containing melon by-product.

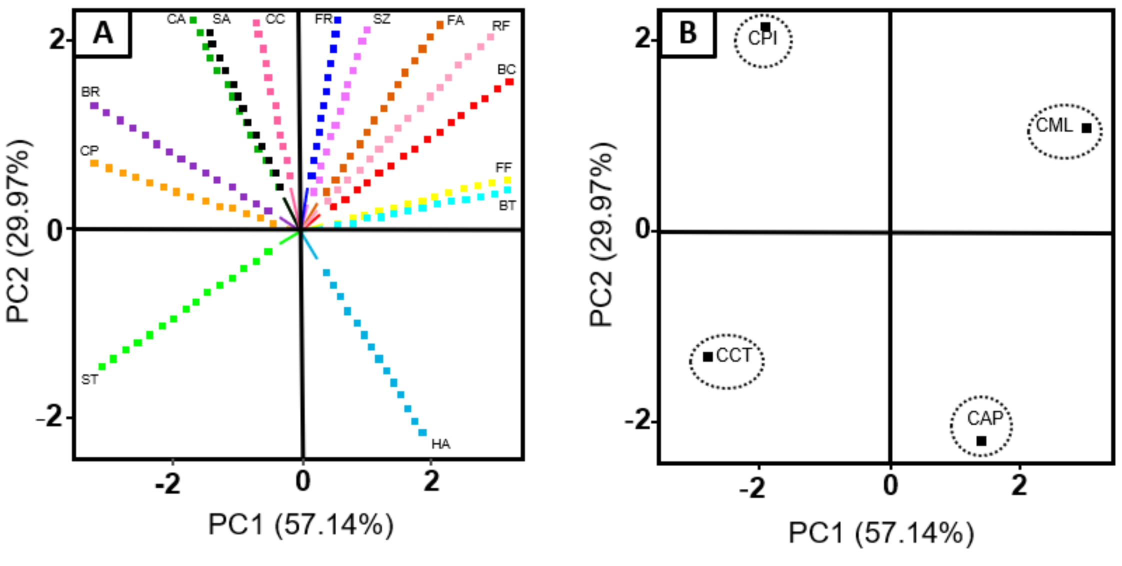

Figure 1. Principal Component Analysis (PCA) using the sensory attributes of cookies: projection of variables (A) and observations (B). PC1 and PC2: Principal Components 1 and 2; BC: brown color, CP: characteristic appearance, SZ: regular size, HA: homogeneous appearance, CA: characteristic aroma, SA: sweet aroma, FA: fruity aroma, ST: sweet taste, BT: bitter taste, FF: fruity flavor, RF: residual flavor, BR: bite resistance, CC: crunchiness, FR: fracturability, CCT: control cookie, CAP: cookie elaborated with wheat flour and 15% of apple by-product, CPI: cookie elaborated with wheat flour and 15% of pineapple by-product, CML: cookie elaborated with wheat flour and 15% of melon by-product.

In the clusters analysis (Figure 2), the observations were separated into four groups according to the cut (0.75) given by the average method. This same cut was used to separate the groups (dotted circles) displayed in the projection of the observations (Figure 1B). Therefore, the cookies formulated with different fruit by-products presented different characteristics between them, and thus, they were separated individually, considering their specificities.

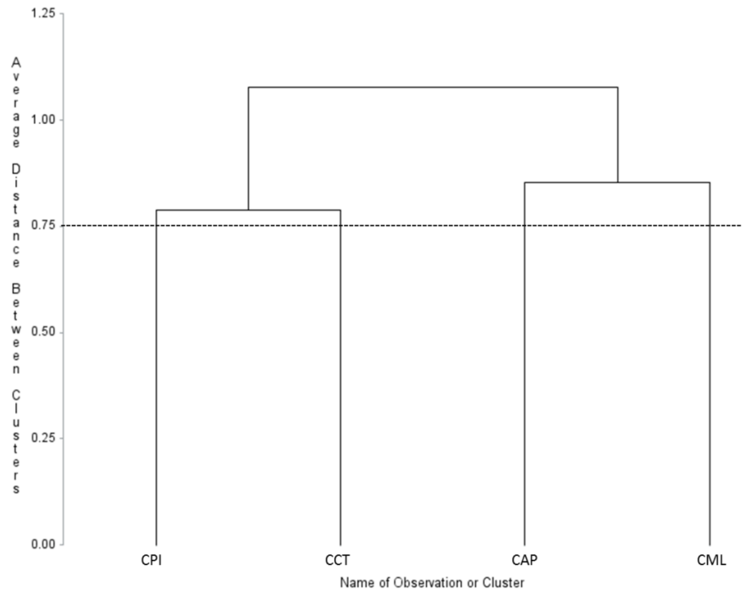

Figure 2. Dendogram obtained from the QDA results of cookies

From these data, it was observed that the CCT presented the lower influence of the attributes bitter taste (BT), residual flavor (RF) and brown color (BC). On the other hand, the CML was the treatment that expressed all these attributes (BF, RF and BC), besides the fracturability (FR), which are considered undesirable for the sensorial quality of cookies. The composition of melon peel is rich in fibers and antioxidant compounds. Factors such as the higher amount of fibers (Toledo *et al*., 2017), and the presence of antioxidants compounds (e.g. polyphenols and flavonoids) (Mallek-Ayadi, Bahloul & Kechaou, 2017) in melon peel can be associated with the accentuated manifestation of these attributes by the CML treatment.

Regarding the texture, it was observed that the CPI presented desirable attributes for cookies such as crunchiness (CC) and bite resistance (BR). The pineapple contains bromelain, which is an enzyme that has a wide range of acting in the food industry, being typically used for the softening of meat products, beer clarification, supplements and bakery products (Bala *et al*., 2012). The bromelain may have influenced the viscosity and texture of cookies dough, causing a larger diameter and lower thickness (Toledo *et al*., 2017) of the cookies, which consequently makes them crunchy and bite resistant (Table 2).

For the CAP, lower relation with aspects of characteristic aroma (CA) and bite resistance (BR) were observed. This result was already expected once a strong negative correlation (r = -0.893) was detected between moisture and hardness parameters, besides that, the CAP reported moisture significantly higher than the CCT and CPI treatments (Table 1). De Camargo *et al*. (2014) developed cookies with 1.8% peanut skin and also stated problems regarding moisture retention and lack of crunchiness. Omoba, Taylor and De Kock (2015) employed red sorghum and millet to elaborate cookies and also observed cookies that were moister and with different aspects of texture when compared to the control (cookie elaborated only with wheat flour).

The fruity aroma (FA) and fruity flavor (FF) were related to the CML, whereas the CCT was the treatment that presented the lowest association with these attributes. This was a predictable observation since CCT formulation did not include ingredients from fruits. According to Yang *et al*. (2012), the melon aroma is formed predominantly by esters, particularly acetic acid phenylmethyl ester. El Hadi *et al*. (2013) have pointed out that some aromatic compounds are linked to sugars such as glycosides, more specifically O-β-D-glycosides and O-diglycosides in the case of fruits, and are released by processing, storage, enzymatic action, acids or heat; as is the case of the cookies, which were baked and stored for 24 hours before being served to the panelists.

Characteristic aroma (CA) and sweet aroma (SA) were highlighted attributes in the CPI treatment. It is known that pineapple is a fruit of ample consumption precisely because of its sweet and attractive aroma. As stated by Zheng *et al*. (2012), the fruit has about 33 compounds responsible for its aroma, especially esters, such as 3-(methylthio)propanoic acid methyl ester and octanoic acid methyl ester which may be associated with these aroma attributes.

In general, it was observed that several studies have been developed aimed at the enrichment of food products with fruit by-products in order to promote the human health and welfare; however, the challenges and technological barriers found in this study were also shared by other authors (Kohajdová, Karovicová & Jurasová, 2013, Omoba, Taylor & De Kock, 2015). Therefore, technological innovations are still needed to subsidize the production and consumption of foods added of by-products, so that they become competitive in the market.

4.4. CONCLUSIONS

The addition of fruit by-products in cookies influenced the sensory profile of the samples. The attributes found in the cookies varied according to the fruit by-product used, and each formulation presented its specificities. Cookies with pineapple by-products showed many desirable attributes for cookies, such as lower moisture, higher crunchiness, bite resistance, sweet aroma and characteristic aroma. Attributes related to the fruit presence (e.g. fruity aroma and flavor) were related to cookies containing melon by-products; however, undesirable characteristics such as fracturability, bitter taste and residual flavor were also described for this formulation. The cookies elaborated with apple by-products presented few desirable attributes and a less complex sensory profile, and it was considered the treatment of worse sensorial quality among the four tested. Thus, it may be concluded that the replacement of wheat flour by fruit by-product modified the appearance, aroma, texture, and flavor of cookies. Due to its physicochemical aspects and sensory profile, the formulation with 15% of pineapple by-product was shown to be more promising for commercial exploitation.

REFERENCES

AOAC Association of Official Analytical Chemists. (2010). *Official methods of analysis of the Association of Official Analytical Chemists*. 18th ed. Gaithersburg, USA: AOAC.

Becker, F.S., Damiani, C., De Melo, A.A.M., Borges, P.R.S. & Boas, E.V.B.V. (2014). Incorporation of buriti endocarp flour in gluten-free whole cookies as potencial source of dietary fiber. *Plants Foods for Human Nutrition,* 69, 344-350.

Bala, M., Ismail, N.A., Mel, M., Jami, M.S., Salleh, H.M. & Amid, A. (2012). Bromelain production current trends and perspective. *Archives des Sciences*, 65, 369-399.

Canalis, M.S.B., Steffonali, M.E., León, A.E., & Ribotta, P.D. (2017). Effect of different fibers on dough properties and biscuit quality. *Journal of the Science of Food and Agriculture*, 97, 1607-1615.

Chadwick, M., Gawthrop, F., Michelmore, R.W., Wagstaff, C. & Methven, L. (2016). Perception of bitterness, sweetness and liking of different genotypes of lettuce. *Food Chemistry*, 197, 66-74.

De Camargo, A.C., Vidal, C.M.M., Canniatti-Brazaca, S.G. & Shahidi, F. (2014). Fortification of cookies with peanut skins: effect on the composition, polyphenols, antioxidant properties, and sensory quality. *Journal of Agricultural and Food Chemistry*, 62, 11228-11235.

El Hadi, M.A.M., Zhang, F.J., Fei-Fei, W., Chun-Hua, Z. & Tao, J. (2013). Advances in fruit aroma volatile research. *Molecules*, 18, 8200-8229.

Ferreira, M. S. L., Santos, M. C. P., Moro, T. M. A., Basto, G. J., Andrade, R. M. S. & Gonçalves, É. C. B. A. (2015). Formulation and characterization of functional foods based on fruit and vegetable residue flour. *Journal of Food Science and Technology*, 52, 822–830.

Gallagher, E., O'Brien, C.M., Scannell, A.G.M. & Arendt, E.K. (2003). Evaluation of sugar replacers in short dough biscuit production. *Journal of Food Engineering*, 56, 261-263.

Handa, C., Goomer, S. & Siddhu, A. (2012). Physicochemical properties and sensory evaluation of frutoligosaccharide enriched cookies. *Journal of Food Science and Technology*, 49, 192-199.

Herken, E.N., Simsek, S., Ohm, J.B. & Yurdunuseven, A. (2017). Effect of mahaleb on cookie quality. *Journal of food processing and preservation*, 41, e13032.

Jayathilakan, K., Sultana, K., Radhakrishna, K. & Bawa, A.S. (2012). Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review. *Journal of Food Science and Technology*, 49, 278-293.

Kohajdová, Z., Karovicová, J. & Jurasová, M. (2013). Influence of grapefruit dietary fibre rich powder on the rheological characteristics of wheat flour dough and on biscuit quality. *Acta Alimentaria*, 42, 91-101.

Mallek-Ayadi, S., Bahloul, N. & Kechaou, N. (2017). Characterization, phenolic compounds and functional properties of Cucumis melo L. peels. *Food Chemistry*, 221, 1691-1697.

Meilgaard, M. C. Civille, G. V. & Carr, B. T. (2006). *Sensory evaluation techniques*. 4. ed. Boca Raton, FL: CRC Press.

Moskowitz, H R. (1983). *Product testing and sensory evaluation of foods*. Westport, USA: Food and Nutrition Press.

Naknaen, P., Itthisoponkul, T., Sondee, A. & Angsombat, N. (2016). Utilization of watermelon rind waste as a potential source of dietary fiber to improve health promoting properties and reduce glycemic index for cookie making. *Food Science and Biotechnology*, 25, 415-424.

Nishibori, S. & Bernhard, R.A. (1993). Model system for cookies: volatile components formed from the reaction of sugar and β-alanine. *Journal of Agricultural and Food Chemistry*, 41, 2374-2377.

Omoba, O.S., Taylor, J.R.N. & de Kock, H.L. (2015). Sensory and nutritive profiles of biscuits from whole grain sorghum and pearl millet plus soya flour with and without sourdough fermentation. *International Journal of Food Science and Technology*, 50, 2554-2561.

Sancho, S.O., Silva, A.R.A., Dantas, A.N.S., Magalhães, T.A., Lopes, G.S., Rodrigues, S., Costa, M.C., Fernandes, F.A.N. & Silva, M.G.V. (2015). Characterization of the industrial residues of seven fruits and prospection of their potential application as food supplements. *Journal of Chemistry*, 1-8, Article ID 264284.

Saponjac, V.T., Cetkovic, G., Canadanovic-Brunet, J., Pajin, B., Djilas, S., Petrovic, J., Loncarevic, I., Stajcic, S. & Vulic, J. (2016). Sour cherry pomace extract encapsulated in whey and soy proteins: Incorporation in cookies. *Food Chemistry*, 207, 27-33.

Siti Roha, A.M., Zainal, S., Noriham, A. & Nadzirah, K.Z. (2013). Determination of sugar content in pineapple waste variety N36. *International Food Research Journal*, 20, 1941-1943.

Sivam, A. S., Sun-Waterhouse, D., Quek, S. & Perera, C. O. (2010). Properties of Bread Dough with Added Fiber Polysaccharides and Phenolic Antioxidants: A Review. *Journal of Food Science*, 75, 163–174.

Sharma, S., Gupta, J. P., Nagi, H. P. S. & Kumar, R. (2012). Effect of incorporation of corn byproducts on quality of baked and extruded products from wheat flour and semolina. *Journal of Food Science and Technology*, 49, 580–586.

Sokal, R. R. & Rohlf, F. J. (1980). *Biometry*. J. Wilson & S. Cotter, Eds. 2nd ed. New York, USA: W.H. Freeman and Company.

Stone, H. & Sidel, J. L. (2004). *Sensory evaluation practices*. 3rd ed. San Diego, USA: Academic Press.

Toledo, N. M. V., Nunes, L. P., Silva, P. P. M., Spoto, M. H. F. & Canniatti-Brazaca, S. G. (2017). Influence of pineapple, apple and melon by-products on cookies: physicochemical and sensory aspects. *International Journal of Food Science & Technology*, 52, 1185–1192.

Van Dyk, J.S., Gama, R., Morrison, D., Swart, S. & Pletschke, B.I. (2013). Food processing waste: Problems, current management and prospects for utilisation of the lignocellulose component through enzyme synergistic degradation. *Renewable and Sustainable Energy Reviews*, 26, 521-531.

Yang, Y., Yu, Zeyuan, Xu, Y. & Shao, Q. (2012). Analysis of volatile compounds from Oriental melons (*Cucumis melo* L.) using headspace SPME coupled with GC-MS. *Advanced Materials Research*, 554-556, 2102-2105.

Zheng, L.Y., Sun, G.M., Liu, Y.G., Lv, L.L., Yang, W.X., Zhao, W.F. & Wei, C.B. (2012). Aroma volatile compounds from two fresh pineapple varieties in China. *International Journal of Molecular Sciences*, 13, 7383-7392.

5. INOSITOL PHOSPHATES AND TOTAL OXALATE OF COOKIES CONTAINING FRUIT BY-PRODUCTS AND THEIR INFLUENCE ON CALCIUM, IRON AND ZINC BIOAVAILABILITY BY CACO-2 CELLS

ABSTRACT

This study aimed to determined inositol phosphates (IP5 and IP6) and oxalate of apple, pineapple, melon by-products and cookies prepared with wheat flour and fruit by-products, besides to evaluate the effect of inositol phosphates (IPs) and oxalate on the bioavailability of calcium, iron and zinc. IPs and oxalate were measured by HPLC-MS/MS. The mineral bioavailability was determined by the molar ratio of phytate and oxalate to minerals and the retention, transport and uptake by Caco-2 cells. Both, phytate and oxalate contents were higher in cookies than in the by-products. The use of pineapple by-product in cookies increased zinc retention (26.40%). Cookies containing melon by-product presented higher uptake for iron (2.14%). For cookies with apple by-product, higher transport (0.06 and 9.71%) and uptake (2.92 and 15.52%) efficiencies were found for calcium and zinc, respectively. In conclusion, the exploitation of fruit by-products could be an alternative to enhance mineral quality of food.

Keywords: Biscuits; Phytate; Oxalic acid; Phytic acid; Antinutrients; Minerals; Uptake; Food waste

5.1. INTRODUCTION

Cookies are important vehicles to increment the nutritional value, besides being versatile and presenting high popularity and acceptance. Many studies have reported the enrichment of cookies with bioactive compounds (e.g. fibers, polyphenols, carotenoids, vitamins) and revealed satisfactory results for technological, nutritional and sensory parameters (Etcheverry, Grusak & Fleige, 2012). However, it is noted that the enrichment of products is not a simple task since the addition of nutrients does not necessarily correspond to a reliable indication of the actual nutritional value of the food, as to admit functionality in the organism the compounds must be available for absorption (Gemede & Ratta, 2014).

In this way, attention must be given to anti-nutritional factors (e.g. phytate, oxalate) that are chemical compounds naturally present in food and/or feedstuffs. They can exert contrary effect to optimum nutrition by different mechanisms (e.g. inactivation of nutrients, decrease of the digestive process or physiological use of food/feed) (Soetan & Oyewole, 2009).

Phytic acid is considered as the major phosporus storage compound in seeds and grains. In the case of cookies made with wheat flour, it is expected that phytate will be one of the most important anti-nutritional compounds present. Inositol phosphates demonstrate a strong affinity to chelate minerals, especially iron, zinc, and calcium. These chelates may originate insoluble salts, which present poor absorption and low bioavailability (Hurrel, 2004).

Oxalic acid occurs ubiquitously in nature, sometimes as free acid, but also as soluble salts (e.g. potassium, sodium or ammonium oxalate) or as insoluble ones (e.g. calcium, iron and magnesium oxalate) (Nguyen & Savage, 2013). Oxalate is considered an anti-nutritional factor that when it is digested, it comes into contact with the nutrients in the gastrointestinal tract, and form oxalic acid binds with nutrients, rendering them inaccessible to the body. Regularly feeding with large amounts of oxalic acid can cause nutritional disorders, as well as severe irritation to the gut (Tadele, 2015). Furthermore, some insoluble salts (e.g. calcium oxalate) have the tendency to precipitate in the kidneys or in the urinary tract, forming crystals that are linked to the formation of kidney stones (Parmar, 2004).

Therefore, the consumption of phytate and oxalate containing foods can result in low bioavailability of minerals, and consequently, anemia, rickets, osteoporosis, and immune diseases that are caused by a deficiency of iron, calcium and zinc (WHO/FAO, 2004). The bioavailability of nutrients has been measured by different methodologies, including both *in vitro* and *in vivo* assays. The simulation of gastrointestinal digestion followed by the Caco-2 cell assay is a well-validated method that has been extensively used to evaluate mineral bioavailability. Caco-2 cells are human intestinal adenocarcinoma cells, which after differentiation, exhibit morphological and functional characteristics of small intestinal absorptive enterocytes (Sambuy *et al.*, 2005).

Thus, this study aimed to investigate the influence of partial replacement of wheat flour by fruit by-products on the inositol phosphates and oxalate content of cookies, as well as to verify the effects of this change on the bioavailability of calcium, iron and zinc. Moreover, this new approach enables an alternative to the use of food by-products, contributing to the nutritional value and mineral bioavailability of cereals products.

5.2. MATERIAL AND METHODS

5.2.1. By-products

By-products of pineapple (central axis), apple (endocarp) and melon (peels) were kindly donated by the industry of minimally-processed foods Della Natural (Engenheiro Coelho, SP, Brazil). The samples were lyophilized and ground by Sublimar Produtos Liofilizados Ltda. (Tatuí, SP, Brazil) and were stored at -18ºC.

5.2.2. Cookies

Cookies were prepared using the same conditions reported by Toledo *et al.* (2017). In total, four formulations were developed, being one the control cookie (CCT), which was prepared only with wheat flour, whereas in the others the wheat flour was partially replaced by 15% of fruit by-products: apple (CAP), melon (CML) and pineapple (CPI). The ingredients used in the cookies formulations were: wheat flour type 1 with 10% of protein (Dona Benta), refined sugar (União), baking powder (Royal), eggs (Qualitá), salted butter (Scala) and pieces of semisweet chocolate bar with 40% of cocoa (Nestlé). All of them were acquired in the local market of Piracicaba, SP, Brazil.

5.2.3. Inositol phosphate content

Inositol phosphates (IPs) were extracted as described by Frontela *et al.* (2009). Samples were added with 0.5 HCl and kept under stirring for 2h. The extracts were centrifuged and the supernatants were frozen overnight. In the next day, the supernatants were transferred into 25 mL volumetric flasks and made up to volume with ultrapure water. Then, it was poured into an anion exchange (SAX) column (500 mg; Supelco, Bellefonte, PA, USA) connected to a vacuum manifold set (20 mmHg). The column was washed with 0.05 M HCl, and then eluted with 2 mL of 2 M HCl. Eluted samples were evaporated to dryness *in vacuo* at 40ºC and dissolved in ultrapure water (1 mL) and tetrabutylammonium hydroxide 40 wt% in water (15 µL) (Sigma Aldrich, 2052-49-5, Switzerland). Inositol phosphate extractions were performed in triplicate.

To determine phytate content, it was considered the sum of IP5 and IP6 since these inositol phosphates present the high binding capacity to minerals. The IPs were determined by HPLC-MS/MS according to Liu, Villalta and Sturla (2009), using reverse-phase chromatography on an Agilent 1100 series (Agilent Technologies, Santa Clara, CA, USA) equipped with a thermostated micro-well plate autosampler and a quaternary pump and connected to an Agilent Ion Trap XCT Plus mass spectrometer (Agilent Technologies) using an electrospray interface (ESI).

Forty microliters of extract were injected into a C18 reverse-phase column (Agilent Technologies) at 40ºC, and eluted at a flow rate of 200 µL/min. The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Inositol phosphates were eluted from 10% to 100% B in 30 min; from 100% to 10% B in 15 min; an isocratic elution of 10% was employed from 45 to 60 min.

The mass spectrometer was operated in negative ion mode with a capillary spray voltage of 3500 V, and a scan speed of 22000 (m/z)/sec from 50–750 m/z. The nebulizer gas pressure, drying gas flow rate and drying gas temperature were set at 30 psi, 8 L/min and 350ºC. Control and data acquisition was performed with Agilent Chemstation Rev B.01.03.SR2. Data were processed using the data analysis software for LC/MSD Trap version 3.3 (Bruker Daltomik, GmbH, Bremen, Germany).

5.2.4. Total oxalate content

Total oxalate extraction was determined according to Savage *et al.* (2000). Briefly, 0.1 g of sample was placed in a 15 mL flask with 5 mL 2 M HCl and incubated in a water bath at 21ºC for 20 min. The extracts were allowed to cool and then transferred quantitatively into 10 mL volumetric flasks and made up to volume. The extracts were centrifuged (Eppendorf, 5804R, Germany) at 5000 rpm for 15 min. The supernatant was filtered through a 0.45 µm cellulose acetate membrane filter (CHMLAB, Barcelona, Spain). Oxalate extractions were performed in triplicate.

The HPLC-MS/MS, working conditions and solvent used were the same as those that used for IP determination, with some modifications. A Supelco Discovery C18 HPLC column (5 µm, 10 x 2.1 mm), thermostated at 40ºC was used, and eluted at a flow of 200 µL/min. Oxalic acid was eluted as follows: an isocratic elution of 10% B was maintained for 5 min; from 10% to 100% B in 25 min. The column was equilibrated at the starting conditions for 10 minutes before each analytical run. Oxalic acid (purified grade, 99.999% trace metals basis, 144-62-7, USA). was used for calibration. Different control samples with known concentrations of oxalic acid (100 to 1 µM) were run in the same conditions.

The mass spectrometer was operated in the same conditions of the IP determinations, but with a scan speed of 22000 (m/z)/sec from 50-150 m/z, with the target mass located at 90 m/z. The Smart ICC target was set to 200.000 counts, whereas the maximum accumulating time was 20 m/secs.

5.2.5. Estimation of relative mineral bioavailability

Molar ratios of antinutrient/mineral were used to estimate mineral bioavailability. The molar ratios were calculated as the millimoles of antinutrient (phytate or oxalate) present in the sample divided by the millimoles of mineral (iron, calcium, and zinc) present in the sample. The critical values used to predict the bioavailability were phytate:calcium > 0.24 (Morris & Ellis, 1985), phytate:iron > 1 (Hurrel, 2004), phytate:zinc > 15 (Turnlund *et al.*, 1984), and oxalate:calcium >1 (Suman & Urooj, 2014).

5.2.6. *In vitro* gastrointestinal digestion

Gastrointestinal digestion was applied to fruit by-products and cookies using the *in vitro* method described by Minekus *et al.* (2014). Simulated Salivary Fluid (SSF), Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) were prepared, and a final ratio of 1:1 (v/v) was targeted at all phases. For the oral phase, 4 g of sample were mixed with 2.5 mL of SSF electrolyte stock solution, 0.5 mL salivary α-amylase (Sigma-Aldrich, catalogue no. A1031) solution of 1500 U/mL, 25 µL of 0.3 M $CaCl_2$ and 975 µL of water. Four parts of oral bolus were mixed with 3 parts of SGF electrolyte stock solution to obtain a final ratio of 1:1 (v/v) after addition of porcine pepsin (Sigma-Aldrich, catalogue no. P-7000), $CaCl_2$ and water. Subsequently, four parts of gastric chyme were mixed with 3 parts of SIF electrolyte stock solution to obtain a final ratio of 1:1 (v/v) after addition of pancreatin (Sigma-Aldrich, catalogue no. P-1750), bile salts (Sigma-Aldrich, catalogue no. B-8756), $CaCl_2$ and water.

5.2.7. Caco-2 cells

The Caco-2 cells assays were carried out according to Frontela *et al.* (2009). Caco-2 cells were obtained from the European Collection of Cell Cultures (ECACC; number 86 010 202, Salisbury, UK) and used in assays at passages 25-38.

Cells were maintained in 75 cm^2 flasks with minimum essential medium (MEM, Gibco BRL Life Technologies, Paisley, Scotland) with 10% v/v heat-inactivated fetal bovine serum (FBS), 1% v/v nonessential amino acids, 1% v/v L-glutamine and 1% antibiotic solution (penicillin-streptomycin) at 37°C in an incubator with 5% CO_2, 95% air atmosphere and 95% relative humidity. After reaching the confluence, the cells were detached with trypsin-EDTA solution and seeded onto polycarbonate membrane chamber inserts (24 mm diameter, 0.4 μm pore size; Transwell, Corning, Inc.) at a density of 50000 cells/cm^2 and allowed to differentiate on filters for 21 days. During this period, cells were maintained in the same conditions above and the medium was changed every 2 days. The experiments were conducted on day 21 from seeding. Microscopic examination of the cultures revealed that confluence was reached after 3-4 days of growth.

Prior to the mineral bioavailability assays, the bioavailable fractions were diluted 1:1 (v/v) with MEM to adjust the osmolality to 280-330 mmol/kg using a vapor pressure osmometer (Wescor, Vapro 5520, USA). The transepithelial electrical resistance (TEER) was measured to verify the integrity of the cell monolayer before and during the experiments (every hour) and monolayers with resistances higher than 500 Ω/cm^2 were used.

5.2.8. Mineral content, solubility, retention, transport, and uptake

The first step of the experiment consisted to remove the growth medium. Phosphate-buffered saline (PBS) at 37°C was used to wash the apical and basolateral cell surfaces. Then, 1 mL of soluble fractions was added to the apical chamber and 1.5 mL of MEM was added to the basal chamber of each cell monolayer. After 4h of incubation, the basal samples were collected. Each experiment was conducted with four replicates. To determine the mineral bioavailability, iron, calcium, and zinc contents were measured in the sample (before digestion), soluble fraction (apical solution), blank (MEM), and in basal solution were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES; ICAP 6500 Duo Thermo-One Fast) following the destruction of organic matter.

The following calculations were used to determine mineral bioavailability: solubility percentages were determined as follows: solubility % = 100 × S/C, where S = soluble mineral content (μg of mineral/g of sample), and C = total mineral content of the sample (μg of mineral/g of sample). The mineral retention was estimated by the difference between mineral soluble added and the mineral amount of the apical and basal chambers after incubation. The retention percentages were calculated by: retention % = 100 × R/C, where R = mineral retention (μg of mineral/well), and C = mineral soluble added (μg). Transport (T) was determined by the difference between the mineral amount in basal chamber of treated samples and the blank. Transport percentages were calculated as transport % = 100 × T/C, where T = cellular transport (μg of mineral/well), and C = mineral soluble added (μg)/well. The cellular uptake was estimated by the sum of retention and transport. Uptake percentage was calculated as the percentage of the mineral added which was taken up by the cells and results were considered as a measure of mineral availability. Due to the differences among samples in terms of the solubility of minerals after *in vitro* digestion, mineral transport and uptake were normalized for solubility as follows: % transport efficiency = (% solubility × % transport)/100, % uptake efficiency = (% solubility × % uptake)/100.

5.2.9. Statistical analyses

Data were expressed as means followed by the standard deviation. The results were subjected to one-way analysis of variances (ANOVA) and the means were compared using the Tukey's test ($p<0.05$). Pearson's correlation analysis was performed to investigate the possible correlation between phytate content; oxalate content; mineral solubility (%); retention (%); uptake (%); and transport (%) by Caco-2 cells. Statistical analyses were performed with the software SAS - Statistical Analysis System, version 9.3 (SAS Institute Inc, Cary, NC, USA).

5.3. RESULTS AND DISCUSSION

5.3.1. Mineral content

Data regarding mineral content are shown in Table 1. In general, the replacement of wheat flour by fruit by-products significantly increased the mineral content of cookies. The melon peels (MLB) presented the highest mineral content for all minerals, and consequently, cookies that contained this by-product (CML) showed higher amounts (mg/100g) of iron (5.05), calcium (239) and zinc (0.97). According to the recommendations of the National Institute of Health (2018), the intake of 100 g of CML would provide 20-24% of calcium; 28-62% of iron; and 9-12% of zinc for women and men between 19-70 years old, respectively. Maintaining an adequate calcium intake may reduce the risk of osteoporosis, osteomalacia, and rickets. The incidence of anemia, and iron deficiency, which may lead to fatigue, and brain disorders, can be decreased by adding iron into the diet. Zinc deficiency, which can lead to problems in the immune system, growth retardation, diarrhea, skin lesions, delayed sexual and bone maturation can also be prevented by zinc intake (WHO/FAO, 2004).

Table 1. Mineral content, inositol phosphates (IP5 and IP6) and total oxalate of fruit by-products and cookies

	Ca	Fe	Zn	IP5	IP6	IP5+IP6	Oxalate
PIB	36.09±0.45^{1c2}	0.84±0.03^{c}	0.67±0.04^{c}	4.23±0.27^{a}	53.80±4.07^{b}	58.04±4.32^{b}	1.82±0.19^{b}
APB	79.82±2.18^{b}	2.39±0.08^{b}	0.99±0.02^{b}	3.83±0.18^{b}	43.63±1.10^{c}	47.47±1.11^{c}	3.02±0.58^{a}
MLB	484.47±10.33^{a}	6.53±0.07^{a}	1.54±0.14^{a}	4.54±0.10^{a}	79.98±3.10^{a}	84.54±3.11^{a}	1.63±0.14^{b}
CCT	119.83±0.30^{c}	3.01±0.15^{c}	0.56±0.05^{c}	193.46±3.99^{a}	271.60±3.57^{a}	465.06±5.10^{a}	5.56±0.37^{a}
CPI	143.18±7.80^{b}	2.98±0.11^{c}	0.68±0.05^{b}	142.65±15.50^{b}	225.92±5.80^{b}	368.57±18.27^{b}	4.06±0.27^{c}
CAP	133.44±7.80^{b}	3.61±0.11^{b}	0.66±0.02^{bc}	116.77±7.73^{c}	194.09±11.57^{c}	310.86±15.31^{c}	4.65±0.23^{b}
CML	239.73±10.22^{a}	5.05±0.14^{a}	0.97±0.04^{a}	163.90±14.77^{b}	219.86±16.14^{b}	383.36±11.66^{b}	3.82±0.15^{c}

[1]Mean±standard deviation (*n*=3). [2]Means followed by different lower case letters within a column show statistically significant differences ($p<0.05$) between the same group of treatments (by-products or cookies). PIB: pineapple by-product; APB: apple by-product; MLB: melon by-product; CCT: cookie control; CPI: cookie containing pineapple by-product; CAP: cookie containing apple by-product; CML: cookie containing melon by-product. Results are expressed in mg/100g.

The use of by-products has been associated with improvements in mineral content of biscuits. Zouari *et al.* (2016) reported significant increases in calcium and zinc contents by the incorporation of sesame peels in cookies. Krishnan *et al.* (2011) added by-products (seed coat) of finger millet on biscuits and found values (mg/100g) between 711 to 864 for calcium, 5.5 to 7.5 for iron, and 2.2 to 2.7 for zinc. Cookies with baru (*Dipteryx alata* Vog.) seeds were elaborated by Caetano *et al.* (2017) and presented values (mg/100g) of 234 for calcium; 21.56 for iron; and 10.85 for zinc, results that were higher than those of our study. Variations in cookies formulations, including the use of different by-products and food processing may be responsible for the discrepancies between the results found (Cilla, López-García & Barberá, 2018).

5.3.2. Phytate and oxalate contents

In addition to mineral content, ascertaining the presence of antinutritional factors is important to mineral bioavailability, since they can compromise the absorption of these elements. Concerning the phytate, cookies showed higher values (310.87 to 465.06 mg/100g) than fruit by-products (47.47 to 84.54 mg/100g), which was expected because phytic acid is the major storage form of phosphorus in cereal products (Frontela *et al.*, 2009). The highest phytate content was observed for the cookie prepared only with wheat flour (CCT) and the lowest for CAP. Similar values were found by Sparvoli *et al.* (2016) who analyzed biscuits prepared with partial replacement of wheat flour by bean flour and found phytate content between 191 to 463 mg/100g. On the contrary, Vitali, Dragojevic and Sebecic (2008) elaborated biscuits with whole grain wheat flour and apple fiber (17%) and identified 530 mg/100g of phytate in tea-biscuits. As expected, this value was higher than the result presented by a similar cookie developed in our study (310.87 mg/100g), containing 15% of apple by-product (CAP). The use of whole grain flours may have contributed to higher phytate contents in these biscuits since whole flours are richer in phytate than white wheat flour (Omoba, Taylor & De Kock, 2015).

Regarding total oxalate, it was noted that cookies also presented higher oxalate contents (3.82 to 5.56 mg/100g) than fruit by-products (1.63 to 3.02 mg/100g). Nevertheless, these amounts were still considered low, which can be interesting from a nutritional point of view. As well as for phytate content, CCT was the treatment that demonstrated the highest amount of oxalate, while CPI and CML presented the lowest values. Literature data showed that total oxalate (mg/100g) may range from 16.8 to 45 for wheat flour; from 6.5 to 14.3 for bakery products, and 18.6 to 22.3 for biscuits (Holmes & Kennedy, 2000; Siener *et al.*, 2006). In the case of cookies, the low oxalate content is probably related to the wheat cultivars used in the flour, cookies formulations and processing effects (Holmes & Kennedy, 2000; Soetan & Oyewole, 2009).

For the by-products, the lowest values of phytate and oxalate were observed for apple (APB) and melon (MLB), respectively. Nitithan, Komindr and Nichachotsalid (2004) analyzed tropical fruits, including pineapple, and demonstrated that phytate values in fruits may range from 37 to 90 mg/100g. In the case of oxalate, values (mg/100g) between 0.6 and 3.5 were found for apple (Holmes & Kennedy, 2000; Ruan *et al.*, 2013); from 1.8 to 8.5 for pineapple (Hernández, Lobo & González, 2009) and 2.7 for melon (Ruan *et al.*, 2013). For both antinutrients, the results of the present study were in agreement with the literature.

It is noted that since fruits as apple, melon and pineapple are not usual sources of phytate and oxalate, the replacement of wheat flour by fruit by-products have the potential to decrease the antinutrients contents in cookies. Vitali, Dragojevic and Sebecic (2008) replaced 17% of wheat flour by apple fibers in biscuits and observed a reduction of 7% in phytate content. In the present study, reductions from 17 to 33% for phytate and from 16 to 31% for oxalate were observed in cookies containing 15% of fruit by-products. This decrease may be interesting because these compounds have strong binding affinity to minerals, forming insoluble salts that compromise the absorption and bioavailability of micronutrients such as calcium, iron and zinc (Gemede & Ratta, 2014).

The mineral bioavailability was estimated by the molar ratio of phytate to calcium, phytate to iron, phytate to zinc and oxalate to calcium (Table 2). According to Kennefick and Cashman (2000), phytate has a more pronounced negative effect on calcium bioavailability than oxalate. However, apparently phytate and oxalate did not compromise Ca bioavailability in samples used in this study since values found were lower than the suggested critical values (> 0.24 for phytate, and >1 for oxalate). Similar results for Phy:Ca were observed by Frontela *et al.* (2009) in infant cereals (0.07 to 0.18); and by Lazaerte *et al*. (2015) for bread (0.03) and wheat flour (0.32). Nevertheless, according to Erba *et al.* (2017), phytate to calcium molar ratio was not a predictive factor of calcium accessibility in ready-to-eat food such as biscuits. Therefore, other assays as Caco-2 cell models could be more efficient for measuring the bioavailability of this mineral. Excepted for melon by-products (MLB), the phytate to iron molar ratios exceeded the critical value (1.0) and may cause interferences in iron bioavailability. Guansheng *et al.* (2005) reported strong phytate interference in the bioavaiability of iron in wheat products and found values up to 26.46 for Phy:Fe. Similar results were found for zinc, since all cookies analyzed showed a Phy:Zn above 15 which may lead to a decrease in the bioavailability of this mineral.

The values found were in agreement with the literature since for wheat flour products these values may be between 0.58 and 80.23 (Guansheng *et al.*, 2005; Lazaerte *et al.*, 2015).

Table 2. Molar ratios of phytate to calcium, iron and zinc and of oxalate to calcium of fruit by-products and cookies

	Phytate:Ca	Phytate:Fe	Phytate:Zn	Oxalate:Ca
PIB	0.07	4.19	6.15	0.02
APB	0.03	1.21	3.40	0.02
MLB	0.01	0.79	3.90	0.00
CCT	0.17	9.38	58.93	0.02
CPI	0.11	7.53	38.58	0.01
CAP	0.10	5.23	33.42	0.02
CML	0.07	4.61	28.07	0.01

PIB: pineapple by-product; APB: apple byproduct; MLB: melon by-product; CCT: cookie control; CPI: cookie containing pineapple by-product; CAP: cookie containing apple by-product; CML: cookie containing melon by-product.

5.3.3. Mineral bioavailability by Caco-2 cells

It has been reported that Caco-2 cell *in vitro* model may contribute to evaluate the mineral bioavailability (Frontela *et al.*, 2009; Vitali *et al.*, 2011; Etcheverry Grusak, & Fleige, 2012). The results obtained for calcium solubility, retention, transport and uptake are presented in Table 3.

Table 3. Calcium solubility, retention, transport and uptake from fruit by-products and cookies

	Solubility (%)	Retention (ug)	Retention (%)	Transport (ug)	Transport efficiency (%)	Uptake (ug)	Uptake efficiency (%)
PIB	32.78±1.05[1a2]	1.04±0.13	0.30±0.0.08[a]	0.72±0.01	0.20±0.00[a]	1.76±0.12	4.53±0.01[a]
APB	34.90±1.95[a]	0.75±0.26	0.18±0.05[b]	0.78±0.15	0.10±0.02[b]	1.53±0.42	1.60±0.43[b]
MLB	12.07±1.71[b]	0.68±0.22	0.27±0.07[b]	0.88±0.06	0.02±0.00[c]	1.56±0.15	2.30±0.61[b]
CCT	29.25±1.18[a]	0.78±0.0.05	0.22±0.01[ab]	0.78±0.09	0.07±0.01[a]	1.56±0.05	1.53±0.12[b]
CPI	17.37±0.90[c]	0.44±0.17	0.18±0.07[ab]	0.61±0.03	0.04±0.00[b]	1.05±0.13	2.42±0.04[a]
CAP	20.73±1.60[b]	0.79±0.28	0.27±0.10[a]	0.85±0.03	0.06±0.01[a]	1.64±0.17	2.92±0.62[a]
CML	15.58±0.17[c]	0.43±0.10	0.11±0.03[b]	0.84±0.03	0.03±0.00[b]	1.27±0.07	2.13±0.12[ab]

[1]Mean±standard deviation (*n*=4). [2]Means followed by different lower case letters within a column show statistically significant differences (p<0.05) between the same group of treatments (by-products or cookies). PIB: pineapple by-product; APB: apple by-product; MLB: melon by-product; CCT: cookie control; CPI: cookie containing pineapple by-product; CAP: cookie containing apple by-product; CML: cookie containing melon by-product.

As shown in Table 3, lower solubility values were recorded for cookies containing fruit by-products. Besides the antinutritional factors, these results may be associated with fiber content of biscuits (Cilla, López-García & Barberá, 2018). In previous assays (Toledo *et al.*, 2017), it was observed that cookies containing fruit by-products demonstrated higher fiber contents than the control. In the same way, other studies confirmed that, depending on the amount and type of fiber, calcium solubility could be affected (Etcheverry, Grusak & Fleige, 2012; Silva *et al.*, 2017). Positive correlation between solubility and transport efficiency (r = 0.850) was observed for calcium. However, the samples with higher solubility not necessarily showed the highest uptake, since moderate negative correlation was found between both these parameters (r =-0.414). The replacement of 15% of wheat flour by pineapple or apple by-product may significantly increase the calcium uptake efficiency. Similar behavior was observed by Vitali *et al.* (2011) for the bioavailability of biscuits enriched with oat fiber.

The values obtained for calcium uptake efficiency varied between 1.53% and 2.92% for cookies, and from 1.60% to 4.53% for by-products. Amongst fruit by-products, pineapple (PIB) showed higher solubility (together with APB), retention, transport and uptake. No data had been provided in the literature on mineral bioavailability of fruits by Caco-2 cells. For cereal products, Vitali *et al.* (2011) reported higher values from calcium uptake of biscuits enriched with pseudo-cereals (from 26.39% to 38.95%), while for infant cereals, Frontela *et al.* (2009) found lower values (between 0.23 and 5.99%) for calcium uptake efficiency. As it was shown by the molar ratios (Table 2), phytate (r = - 0.385) and oxalate (r = -0,484) did not strongly correlate with calcium uptake efficiency. Etcheverry, Grusak and Fleige (2012) confirm that other factors (e.g. presence of dietary fibers, Maillard reaction products, organic acids, and calcium salts) should be considered to determine calcium bioavailability.

The results obtained for iron bioavailability are summarized in Table 4. Amongst by-products, the highest solubility percentage was found for pineapple (PIB). Residues of orange and lime were analyzed by Silva *et al.* (2017) and significant amounts of soluble iron (54.99% and 19.36%, respectively) were also found. In relation to the other parameters of bioavailability, PIB also presented the highest transport and uptake efficiencies values, which was expect, since pineapple usually has large amounts of organic acids (malic and citric acids) (Hernández, Lobo & González, 2009) that may contribute to iron absorption (Etcheverry, Grusak & Fleige, 2012).

Table 4. Iron solubility, retention, transport and uptake from fruit by-products and cookies

	Solubility (%)	Retention (ug)	Retention (%)	Transport (ug)	Transport efficiency (%)	Uptake (ug)	Uptake efficiency (%)
PIB	37.25±3.46[1a2]	0.20±0.01	6.92±0.80[c]	0.22±0.05	2.75±0.73[a]	0.42±0.04	5.31±0.55[a]
APB	6.72±0.51[c]	0.36±0.05	22.08±1.08[a]	0.16±0.05	0.65±0.23[b]	0.52±0.11	2.14±0.45[b]
MLB	10.37±0.57[b]	0.70±0.08	10.29±0.46[b]	ND	ND	0.70±0.08	1.07±0.13[c]
CCT	12.89±0.47[a]	0.41±0.01	10.58±0.01[a]	ND	ND	0.41±0.01	1.36±0.00[c]
CPI	11.68±0.29[c]	0.20±0.04	5.81±0.69[c]	ND	ND	0.20±0.04	0.68±0.11[d]
CAP	12.05±0.41[bc]	0.35±0.03	7.75±0.25[b]	0.29±0.01	0.80±0.04[a]	0.64±0.04	1.71±0.05[b]
CML	12.53±0.44[ab]	0.61±0.04	10.00±0.28[a]	0.43±0.03	0.86±0.07[a]	1.04±0.08	2.14±0.09[a]

[1]Mean±standard deviation (*n*=4). [2]Means followed by different lower case letters within a column show statistically significant differences (p<0.05) between the same group of treatments (by-products or cookies). PIB: pineapple by-product; APB: apple by-product; MLB: melon by-product; CCT: cookie control; CPI: cookie containing pineapple by-product; CAP: cookie containing apple by-product; CML: cookie containing melon by-product.

For cookies, significant differences were observed indicating that the addition of fruit by-products tends to decrease the iron solubility percentages. As observed by Frontela *et al.* (2009), positive correlation was found between iron solubility with cell transport (r = 0.879) and uptake (r = 0.890) efficiencies. Considering that these parameters can be used as bioavailability predictors, it can be stated that even with the highest fiber contents (Toledo *et al.*, 2017), the treatments CML and CAP demonstrated greater iron bioavailability than CCT (control). No strong correlations were observed between iron uptake efficiency with phytate (r = -0.493) or oxalate (r = -0.469) contents. It suggests that some other compounds such as proteins (e.g. egg yolk), calcium, zinc, manganese, and tannins, probably may have acted as inhibitors of iron absorption (Etcheverry, Grusak & Fleige, 2012).

Otherwise, strong positive correlation was observed between solubility and retention (r = 0.982) for cookies. Therefore, as for solubility, no significant differences were observed between CCT and CML for iron retention. The transport values for CCT and CPI could not be detected due to their small amounts. Other authors reported retention, transport and uptake efficiencies higher than those found in our study. Frontela *et al.* (2009) stated values between 0.66 and 6.05 for uptake efficiency in infant cereals and attributed the increased of iron bioavailability due to previous phytate treatment. On the other hand, Vitali *et al.* (2011) improved iron bioavailability of biscuits adding pseudo-cereals and reported values from 9.53% to 13.99% in biscuits containing carob and apple fiber, respectively.

Zinc solubility, retention, transport and uptake efficiencies can be observed in Table 5. Differently of the other minerals analyzed, negative correlations were found between solubility with transport (r = -0.516) and uptake (r = -0.662) efficiencies. Solubility percentage (%) of by-products ranged from 44.48 to 57.91, with higher value for APB, followed by MLB and PIB.

Table 5. Zinc solubility, retention, transport and uptake from fruit by-products and cookies

	Solubility (%)	Retention (ug)	Retention (%)	Transport (ug)	Transport efficiency (%)	Uptake (ug)	Uptake efficiency (%)
PIB	52.21±0.46[1b2]	0.14±0.05	4.00±1.12[a]	0.27±0.05	4.06±0.54[a]	0.41±0.00	6.14±0.05[ab]
APB	57.91±2.08[a]	0.48±0.23	8.12±2.74[a]	0.25±0.00	2.52±0.04[b]	0.73±0.23	7.34±1.66[a]
MLB	44.48±3.32[c]	0.28±0.10	3.88±0.96[a]	0.37±0.03	2.42±0.18[b]	0.65±0.06	4.24±0.27[b]
CCT	45.76±3.71[a]	0.48±0.06	20.23±1.69[b]	0.16±0.05	3.01±0.68[b]	0.64±0.00	11.91±0.23[bc]
CPI	35.94±0.25[b]	0.65±0.07	26.40±1.96[a]	0.20±0.05	2.86±0.55[b]	0.85±0.12	12.39±1.25[b]
CAP	31.04±0.73[c]	0.38±0.11	18.39±3.61[b]	0.64±0.03	9.71±0.33[a]	1.02±0.08	15.52±2.33[a]
CML	33.51±0.64[bc]	0.64±0.02	19.88±0.59[b]	0.32±0.08	3.30±0.62[b]	0.96±0.10	9.88±0.76[c]

[1]Mean±standard deviation (n=4). [2]Means followed by different lower case letters within a column show statistically significant differences (p<0.05) between the same group of treatments (by-products or cookies). PIB: pineapple by-product; APB: apple by-product; MLB: melon by-product; CCT: cookie control; CPI: cookie containing pineapple by-product; CAP: cookie containing apple by-product; CML: cookie containing melon by-product.

Regarding cookies, the solubility (%) was between 31.04 and 45.76, and as observed for iron, the treatments containing fruit by-products had lower solubility values, and CAP presented the lowest one. Over again, it was noted that the presence of fruit by-products in cookies decreased mineral solubility. However, it did not impact on the zinc bioavailability, since the treatment CAP was the one that obtained the highest transport and uptake efficiencies. No differences were observed for the retention (%) of fruit by-products, neither for cookies (except by CPI). Regarding the transport efficiency, greater amounts of zinc were found for PIB, amongst by-products, and CAP, for cookies. Higher values of zinc uptake (%) were reported by Viadel, Barberá and Farré (2006) for cooked beans (17.21) and by Frontela *et al.* (2009) for infant cereals (from 5 to 41.6). In this study, phytate and oxalate content demonstrated considerable negative effect on the zinc uptake efficiency (r = -0.771 and r = -0,877) which has probably compromised its bioavailability.

5.4. CONCLUSIONS

It was noted that phytate was the prevalent antinutrient in both fruit by-products and cookies. In general, the mineral solubility decreased in biscuits with the addition of fruit by-products. However, it did not necessarily interferer in the bioavailability of minerals. As predicted by the molar ratios, the antinutrients compromised the bioavailability of iron and zinc but did not affect the bioavailability of calcium. In the case of iron, better bioavailability results (retention, transport and uptake efficiencies) were observed for the cookies containing melon by-products. It was verified that the replacement of wheat flour by pineapple by-product increased the retention of zinc. The use of apple by-product seems to be also promising due to the high transport and uptake efficiencies found both for calcium and for zinc. Therefore, it was concluded that the use of fruit by-products in cookies can be interesting to reduce the antinutrients content (phytate and oxalate), besides contributing with the increase of the bioavailability of minerals such as calcium, iron and zinc, depending on the by-product used.

REFERENCES

Caetano, K.A., Ceotto, J.M., Ribeiro, A.P.B., Morais, F.P.R., Ferrari, R.A., Pacheco, M.T.B. & Capitani, C.D. (2017). Effect of baru (Dipteryx alata Vog.) addition on the composition and nutritional quality of cookies. *Food Science and Technology*, 37, 239-245.

Cilla, A., López-García, G. & Barberá, R. (2018). *In vitro* bioavailability of iron and calcium in cereals and derivatives: A review. *Food Reviews International*, 34, 1-33.

Erba, D., Manini, F., Meroni, E. & Casiraghi, M. C. (2017). Phytate/calcium molar ratio does not predict accessibility of calcium in ready-to-eat dishes. *Journal of the Science of Food and Agricultural*, 97, 3189–3194.

Etcheverry, P., Grusak, M.A. & Fleige, L.E. (2012). Application of in vitro bioaccessibility and bioavailability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc, and vitamins B6, B12, D, and E. *Frontiers in Physiology*, 3, 1-22.

Frontela, C., Scarino, M.L., Ferruzza, S., Ros, G. & Martínez, C. (2009). Effect of dephynization on bioavailability of iron, calcium and zinc from infant cereals assessed in the Caco-2 cell model. *World Journal of Gastroenterology*, 15, 1977-1984.

Gemede, H.F. & Ratta, N. (2014). Antinutritional factors in plant foods: Potential health benefits and adverse effects. *International Journal of Food Science and Nutrition*, 3, 284-289.

Guansheng, M.A., Jin, Y., Piao, J., Kok, F., Guusje, B. & Jacobsen, E. (2005). Phytate, calcium, iron, and zinc contents and their molar ratios in foods commonly consumed in China. *Journal of Agricultural and Food Chemistry*, 53, 10285-10290.

Hernández, Y., Lobo, M.G. & González, M. (2009). Factors affecting sample extraction in the liquid chromatographic determination of organic acids in papaya and pineapple. *Food Chemistry*, 114, 734-741.

Holmes, R.P. & Kennedy, M. (2000). Estimation of the oxalate content of foods and daily oxalate intake. *Kindey International*, 57, 1662-1667.

Hurrel, R.F. (2004). Phytic acid degradation as a means of improving iron absorption. International *Journal for Vitamin and Nutrition Research*, 74, 445-452.

Kennefick, S. & Cashman, K.D. (2000). Investigation of an in vitro model for predicting the effect of food components on calcium availability from meals*. International Journal of Food Science and Nutrition*, 51, 45-54.

Krishnan, R., Dharmaraj, U., Manohar, R.S. & Malleshi, N.G. (2011). Quality characteristics of biscuits prepared from finger millet seed coat based composite flour. *Food Chemistry*, 129, 499-506.

Lazaerte, C.E., Nils-Gunnar, C., Almgren, A., Sandberg, A.S. & Granfeldt, Y. (2015). Phytate, zinc, iron and calcium content of common Bolivian food, and implications for mineral bioavailability. *Journal of Food Composition and Analysis*, 39, 111-119.

Liu, X., Villalta, P.W. & Sturla, S.J. (2009). Simultaneous determination of inositol and inositol phosphates in complex biological matrices: quantitative ion-exchange chromatography/tandem mass spectrometry. *Rapid Communications in Mass Spectrometry*, 23, 705-712.

Minekus, M. Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D.J., Ménard, O., Recio, I., Santos, C.N., Singh, R.P., Vegarud, G.E., Wickham, M.S., Weitschies, W. & Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food - an international consensus. *Food & Function*, 5,1113-1124.

Morris, E. R. & Ellis, R. (1985). Bioavailability of dietary calcium: Effect of phytate on adult men consuming nonvegetarian diets. In C. Kies (Ed.), *Nutritional Bioavailability of Calcium*, Washington: American Chemical Society.

National Institute of Health. (2018). *Dietary Reference Intakes (DRIs): Recommended Dietary Allowance and Adequate Intakes, Elements*. https://www.ncbi.nlm.nih.gov/books/NBK56068/table/summarytables.t3/?report=objectonly. Accessed 23 April 2018.

Nguyen, H.V.H. & Savage, G.P. (2013). Oxalate content of New Zealand grown and imported fruits. *Journal of Food Composition and Analysis*, 31, 180-184.

Nitithan, S., Komindr, S. & Nichachotsalid, A. (2004). Phytate and fiber content in Thai fruits commonly consumed by diabetic patients. *Journal of the Medical Association of Thailand*, 87, 1444-1446.

Omoba, O.S., Taylor, J.R.N. & de Kock, H.L. (2015). Sensory and nutritive profiles of biscuits from whole grain sorghum and pearl millet plus soya flour with and without sourdough fermentation. *International Journal of Food Science and Technology*, 50, 2554-2561.

Parmar, M.S. Kidney stones. (2004). *British Medical Journal*, 328, 1420–1424.

Ruan, Q.Y., Zheng, X.Q., Chen, B.L., Xiao, Y., Peng, X.X., Leung, D.W.M. & Liu, E.E. (2013). Determination of total oxalate contents of a great variety of foods commonly available in Southern China using an oxalate oxidase prepared from wheat bran. *Journal of Food Composition and Analysis*, 32, 6-11.

Sambuy, Y., De Angelis, I., Ranaldi, G., Scarino, M.L., Stammati, A. & Zucco, F. (2005). The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. *Cell Biology and Toxicology*, 21, 1-26.

Savage, G.P., Vanhanen, L., Mason, S.M. & Ross, A.B. (2000). Effect of cooking on the soluble and insoluble oxalate content on some New Zealand food. *Journal of Food Composition and Analysis*, 13, 201-206.

Siener, R., Honow, R., Voss, S., Seidler, A. & Hesse, A. (2006). Oxalate content of cereals and cereal Products. *Journal of Agricultural and Food Chemistry*, 54, 3008-3011.

Silva, J.G.S., Rebellato, A.P., Greiner, R. & Pallone, J.A.L. (2017). Bioaccessibility of calcium, iron and magnesium in residues of citrus and characterization of macronutrients. *Food Research International*, 97, 162-169.

Soetan, K.O. & Oyewole, O.E. (2009). The need for adequate processing to reduce the antinutritional factors in plants used as human foods and animal feeds: A review. *African Journal of Food Science*, 3, 223-232.

Sparvoli, F., Laureati, M., Pilu, R., Pagliarini, E., Toschi, I., Giuberti, G., Fortunati, P., Draminati, M.G., Cominelli, E. & Bollini, R. (2016). Exploitation of common bean flours with low antinutrient content for making nutritionally enhanced biscuits. *Frontiers in Plant Science*, 7, 1-14.

Suman, P.F. & Urooj, A. (2014). Nutrients, antinutrients and bioacessible mineral content (in vitro) of pearl millet as influenced by milling. *Journal of Food Science and Technology*, 51, 756-761.

Tadele, Y. (2015). Important anti-nutritional substances and inherent toxicants of feeds. *Food Science and Quality Management*, 36, 40-48.

Toledo, N.M.V., Nunes, L.P., Silva, P.P.M., Spoto, M.H.F. & Canniatti-Brazaca, S.G.C. (2017). Influence of pineapple, apple and melon by-products on cookies: physicochemical and sensory aspects. *International Journal of Food Science and Technology*, 52, 1185-1192.

Turnlund, J. R., King, J. C., Keyes, W. R., Gong, B. & Michel, M. C. (1984). A stable isotope study of zinc absorption in young men: Effects on phytate and R-cellulose. *American Journal of Clinical Nutrition, 40*, 1071-1077.

Viadel, B., Barberá, R. & Farré, R. (2006). Calcium, iron and zinc uptakes by Caco-2 cells from white beans and effect of cooking. *International Journal of Food Science and Nutrition*, 57, 190-197.

Vitali, D., Dragojevic, I.V. & Sebecic, B. (2008). Bioaccessibility of Ca, Mg, Mn and Cu from whole grain tea-biscuits: impact of proteins, phytic acid and polyphenols. *Food Chemistry*, 110, 62-68.

Vitali, D., Radic, M., Cetina-Cizmek, B. & Dragojevic, V. (2011). Caco-2 cell uptake of Ca, Mg, and Fe from biscuits as affected by enrichment with pseudocereal/inulin mixtures. *Acta Alimentaria*, 40, 480-489.

Zouari, R., Besbes, S., Ellouze-Chaabouni, S. & Ghribi-Aydi, D. (2016). Cookies from composite wheat-sesame peels flours: dough quality and effect of Bacillus subtilis SPB1 biosurfactant addition. *Food Chemistry*, 94, 758-769.

WHO/FAO. World Health Organization and Food and Agriculture Organization of the United Nations. (2004). *Vitamin and mineral requirements in human nutrition*. 2nd ed. Geneva: WHO/FAO.

6. GENERAL CONCLUSIONS

In general, pineapple, apple and melon by-products demonstrated significant amounts of phenolic compounds, fibers and minerals, as well as physicochemical (color, water activity, pH, agrochemical residues) and microbiological properties appropriate for their use as ingredients in new food formulations.

In the present study, the application of fruit by-products was suggested for the preparation of cookies and different behaviors were verified for the formulations in relation to the by-products employed: cookies containing 15% of melon by-products were more prominent regarding the nutritional aspects, presenting satisfactory amounts of dietary fibers and minerals. On the other hand, cookies with 15% of pineapple by-products were sensorially prominent for presenting a high acceptance and purchase intention. Formulations containing 15% of apple by-product, in turn, demonstrated a higher content of total phenolics and a higher antioxidant capacity.

In general, the use of fruit by-products reduced the contents of antinutritional factors such as phytates and oxalates and contributed to mineral bioavailability in cookies, with pineapple by-product positively influencing zinc retention, whereas melon by-product presented a relevant iron absorption and apple by-product contributed to the transport efficiency and calcium and zinc absorption, respectively.

Considering the results found, it was observed that the fruit by-products demonstrated a relevant contribution for the nutritional, technological and sensory aspects in cookies. It is expected that the present approach might encourage the use of by-products in the development of new products aimed at human consumption, attributing to these ingredients a higher added value, and avoiding their improper disposal.