ELÍDIO ZAIDINE MAURÍCIO ZITHA

DEVELOPMENT OF CHICKEN NUGGETS AND MEAT ANALOGUE NUGGETS OF CHICKPEA (*Cicer arietinum* L.) WITH ADDED NUTRITIONAL AND FUNCTIONAL VALUE BY PEQUI (*Caryocar brasiliense* Camb.) PULP FLOUR

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência dos Alimentos, área Ciência dos Alimentos, para a obtenção do título de Doutor.

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DESENVOLVIMENTO DE EMPANADOS DE FRANGO E ANÁLOGOS À CARNE À BASE DE GRÃO-DE-BICO (*Cicer arietinum* L.) COM VALOR NUTRICIONAL E FUNCIONAL AGREGADO POR FARINHA DE POLPA DE PEQUI (*Caryocar brasiliense* Camb.)

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência dos Alimentos, área Ciência dos Alimentos, para a obtenção do título de Doutor.

APROVADA em 01 de fevereiro de 2023.

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2023

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GENERAL ABSTRACT

The growing consumer awareness about the relationship between good quality food and a healthy life, together with the rapid world population growth, stimulates the development of functional meat products and the search for alternative protein sources to meet the growing demand for meat. In this context, several meat products and meat alternatives have been developed using functional ingredients. The Brazilian Cerrado presents a wide range of native species rich in bioactive compounds, whose fruits can be incorporated into the preparation of functional products, in order to meet the growing demand for healthy eating, as well as to preserve the biome and valuing the small producer. Among the native Cerrado fruits, the pequi stands out for its high nutritional value, peculiar flavor and aroma, and functional appeal. This study aimed to add value to the fruit of the Cerrado, through the elaboration of chicken nuggets and chickpea meat analogue (CMA) nuggets formulated with different concentrations of pequi and submitted to different cooking methods during storage time. For both types of nuggets, the incorporation of pequi flour promoted an increase in cooking yield, texture profile parameters (hardness, springiness, cohesiveness and chewiness), fiber content, lipids, carotenoids, monounsaturated fatty acid (MUFA)s, saturated fatty acids (SFA), terpenes and esters, and reduced the content of polyunsaturated fatty acids (PUFA). The TBARS values for the chicken nuggets increased significantly with increasing the concentration of pequi flour and over storage time. As for chickpea meat analogue, the TBARS values also increased with the increase of pequi flour, however, decreased over storage time. For chicken nuggets, air fryer resulted in higher cooking yield and carotenoids. For both types of nuggets, deep frying exhibited higher polyunsaturated and monounsaturated fatty acid contents and lower saturated fatty acid values. Although the TBARS levels increased, the values were within acceptable limits. In both cases, deep frying w had the highest sensory acceptability, being chosen as the best cooking method for chicken nuggets and chickpea meat analogues nuggets. Inclusion of pequi flour up to 9% can be recommended in the formulation of chicken nuggets and chickpea meat analogue nuggets without affecting the sensory properties. The results confirmed the potential of pequi flour as a promising ingredient in meat and meat analogues products.

Keywords: Functional food. Dietary fiber. Carotenoids. *Caryocar Brasiliense*. Nugget. *Cicer arietinum*

RESUMO GERAL

O crescente aumento da conscientização do consumidor sobre a relação entre uma alimentação de boa qualidade e uma vida saudável, aliado ao rápido crescimento da população mundial, estimula o desenvolvimento de produtos cárneos funcionais e a busca de fontes proteicas alternativas para fazer face à crescente demanda de carne. Neste contexto, diversos produtos cárneos e alternativos à carne têm sido elaborados utilizando ingredientes funcionais. O Cerrado brasileiro apresenta uma vasta gama de espécies nativas ricas em compostos bioativos, cujos seus frutos podem ser incorporados na elaboração de produtos funcionais, com vista a fazer face à crescente demanda por alimentação saudável, bem como a preservação do bioma e valorização do pequeno produtor. Dentre os frutos nativos do Cerrado, destaca-se o pequi, pelo seu alto valor nutricional, sabor e aroma peculiares e apelo funcional. Esse estudo teve como objetivo agregar valor a frutos do Cerrado, através da elaboração de empanados de frango e a base de grão-de-bico formulados com diferentes concentrações de pequi e submetidos a diferentes métodos de cocção durante o tempo de armazenamento. Para os dois tipos de empandos, a incorporação de farinha de pequi promoveu um aumento no rendimento de cocção, parâmetros de perfil de textura (dureza, elasticidade, coesividade e mastigabilidade), teores de fibras, lipídios, carotenoides, ácidos graxos monoinsaturados (MUFA), ácidos graxos saturados (SFA), terpenos e ésteres, e reduziu os teores de ácidos graxos poli-insaturados (PUFA). Os valores de substâncias reativas ao ácido tiobarbitúrico (TBARS) para os empanados de frango aumentaram significativamente com o aumento de concentração de farinha de pequi e ao longo do tempo de armazenamento. Já para os empanados a base de grão-de-bico, os valores de TBARS também aumentaram com o aumento de farinha de pequi, mas ao longo do tempo registaram uma queda. Para empanados de frango, a cocção por air-fryer resultou em maior retenção de carotenoides e maior rendimento. Para os dois tipos de empanados, a fritura em óleo revelou teores mais altos de ácidos graxos poli-insaturados e monoinsaturados e valores mais baixos de ácidos graxos saturados. Apesar de ter sido observado um aumento nos níveis de TBARS, os valores estiveram dentro dos limites aceitáveis. Nos dois casos, a fritura em óleo resultou em uma maior aceitação sensorial, tendo sido escolhido como o melhor método de cocção. A incorporação de farinha de pequi até 9% pode ser recomendada na formulação de empanados de frango e a base de grão-de-bico sem afetar as características sensoriais. Os resultados confirmaram o potencial de farinha de pequi como um ingrediente promissor em produtos cárneos e a análogos de carne.

Palavras-chave: Alimento funcional. Fibra dietética. Carotenoides. *Caryocar Brasiliense*. Empanado. *Cicer arietinum* L

SUMMARY

FIRST SECTION

1 GENERAL INTRODUCTION

In recent years, consumer demand for fast, convenient, and healthy food has increased significantly due to changes in lifestyle (POURASHOURI *et al*., 2021). Among the existing options, meat is a versatile food. Meat and meat products are good sources of high-quality protein in the global human diet. It is a source of high biological value proteins, essential vitamins (B6 and B12), essential amino acids, and minerals such as iron and zinc that are efficiently absorbed by the human intestine (CABRERA; SAADOUN, 2014; GRASSO *et al*., 2022).

Despite its high nutritional value, the consumption paradigm of meat and meat products has been modified with the information disseminated by media about the quality and healthiness of meat products, resulting in various concerns about their consumption (ALONSO; GONZÁLEZ-MONTAÑA; LOMILLOS, 2020; ANDRADE *et al*., 2017; COOREMAN-ALGOED *et al*., 2022). Certain compounds usually found in meat product, including saturated fatty acids, cholesterol, salt, and other additives, have been associated with increased risk of non-transmissible chronic diseases, including cardiovascular issues, metabolic diseases diabetes, and cancer (DOMINGO; NADAL, 2017; HAN; BERTRAM, 2017; THØGERSEN; BERTRAM, 2021).

Moreover, considering the facts that meat is deficient in essential dietary fibers and bioactive compounds such as phenolics and carotenoids, consumers began to worry about the food quality, mainly due the current lifestyle that prioritizes healthier meat products. In this regard, several studies have been carried out in recent years aiming to develop meat products with improved and healthier compositions by incorporating functional ingredients which are considered beneficial for health thus reducing the risks of harmful effects (BIS-SOUZA *et al*., 2019; POGORZELSKA-NOWICKA *et al*., 2018; URSACHI; PERȚA-CRIȘAN; MUNTEANU, 2020).

The reformulation of meat products using fruits and vegetables is considered to be an attractive strategy to increase their healthiness, as these plant foods are excellent source of dietary fiber and bioactive compounds with functional properties and potential industrial applications (PANWAR *et al*., 2021).

In the other hand, with a rapidly growing global population, which is projected to reach 9 billion people by the year 2050 (RUBIO; XIANG; KAPLAN, 2020), there have been increasing global studies on plant-based meat analog as an alternative protein source to reduce the widening gaps between traditional meat production and its higher consumer demands in the near future (SAKAI *et al*., 2022). Furthermore, environmental and ethical issues related to animal welfare and slaughter as well as health issues associated with red meat are prevailing concerns surrounding meat products (DEKKERS; BOOM; VAN DER GOOT, 2018). These concerns along with the shift towards vegan and vegetarian dietary habits due to psychosocial beliefs such as social status, religion, ideologies have led to interest in the production of plant-based meat analogue.

Therefore, due to researchers' pursuit for bioactive compounds to formulate functional meat products and plant-based meat analogues products, many plants species from Brazilian Cerrado owing to their high nutritional and functional properties have been extensively studied. Among these fruits, pequi (*Caryocar Brasiliense* Camb.) stands out for being rich in lipids, especially oleic and palmitic fatty acids, and for having high levels of fiber, vitamin C, carotenoids and phenolic compounds, which gives it a high antioxidant capacity (LEÃO *et al*., 2017; RODRIGUES *et al*., 2015).

Chickpea (*Cicer arietinum* L.) is a widely consumed pulse in the world. Is an excellent source of carbohydrates, high-quality protein and dietary fiber along with micronutrients (KAUR; PRASAD, 2021). Chickpea proteins find a wide range of applications in the food sector owing to their low cost, well balanced amino acid content and good digestibility in comparison to other pulses proteins (BOUKID, 2021; FARIDY *et al*., 2020). Moreover, chickpea proteins have good functional properties, including water holding, solubility, emulsifying, foaming and gelling capacities (SHAABANI *et al*., 2018; SOFI *et al*., 2020). Theses technological properties make chickpea as good alternative to conventional meat-based foods.

Therefore, the objective of this study was to develop and characterize chicken nuggets and chickpea meat analogues formulated with the incorporation of pequi flour in order to supply the consumers' demand for healthy food and also adding value to this fruit and reduce the possible environmental impacts in Brazilian Cerrado.

2 THEORETICAL REFERENCE

2.1 Functional meat products

In the last decades, animal proteins from meat products have been considered as of the main concerns to achieve a sustainable food production (ROCCHETTI *et al*., 2023). Meat is one of the most nutritious food globally consumed due to its high biological value protein in terms of well-balanced amino acid composition, along with vitamin B12, vitamin D, and minerals such as iron, zinc and selenium. In addition, meat products are highly appreciated by the consumers for their sensory characteristics (ASGAR *et al*., 2010).

Despite its nutritional value meat is deficient in dietary fiber (DAS *et al*., 2020) and contains high levels of cholesterol (MAFRA *et al*., 2018). Furthermore, some studies have appointed that intake of high-fat processed meat products can be related with significant risk of some diseases such as cardiovascular and metabolic diseases and cancer (DOMINGO; NADAL, 2017; HAN; BERTRAM, 2017). In this sense, consumer demand for healthier food has drastically increased due to the awareness of the correlation between a healthy lifestyle and dietary habits with reduced incidence of such diseases.

Due to these concerns, over the last two decades, food industry has started to research for health-beneficial ingredients to develop functional meat products that attract economic commodities (DIAS; FERREIRA; BARREIRO, 2015). Functional foods are those that beyond the intrinsic nutritive effect may also play a positive role in specific biological functions, improving the general state of health and lower the risk of suffering certain type diseases (RODRÍGUEZ *et al*., 2006; ROMERO *et al*., 2019).

The reformulation of meat products to develop healthier and more sustainable foods can be seen as an excellent strategy, in line with the Sustainable Development Goals proposed by the United Nations, appearing also to be aligned with the need to reduce the association of meat and processed meat to colon cancer (KRUGER; ZHOU, 2018; QIAN *et al*., 2020). Within this scenario, the incorporation of plant-based ingredients into meat products has been proven as a successful and consumer-accepted strategy to promote a healthier diet, without requiring the consumers to change their eating habits (CARPENTIERI *et al*., 2022; LANG, 2020).

Incorporation of dietary fibers into meat products has increased over the last decades owing to their positive effects of potentially inhibiting breast and colon tumor growth, reducing the risk of coronary heart diseases, preventing cholesterol and lowering obesity risk (

HATHWAR *et al*., 2012). Apart from this, dietary fibers owing to their functional properties help to improve various quality parameters of meat products, such as water holding capacity, emulsion stability, cooking yield, juiciness, shrinkage and rheological properties. As a result of these functional properties, various dietary fibers alone or in combination have been extensively assessed to substitute fat in meat products to change the health attributes and maintain the desirable texture properties (AMARAL *et al*., 2015; GARCÍA *et al*., 2017; GARCÍA; CÁCERES; DOLORES SELGAS, 2006; GIBIS; SCHUH; WEISS, 2015; HAN; BERTRAM, 2017; HENNING; TSHALIBE; HOFFMAN, 2016; KEHLET et al., 2017; OZ *et al*., 2016). It has been reported that intake of fiber rich foods decreases the risk of cardiovascular disease, colon cancer and obesity (HAN; BERTRAM, 2017). Additionally, fruits and vegetables rich in phenolic compounds are suitable for application in meat products. The antioxidant activity of these compounds is seen to be the main advantage of their application, promoting an increase in shelf life of these foods conferring health benefits to consumers (LORENZO *et al*., 2018). The most recent studies about the incorporation of plant-based sources in the meat products are summarized in Table 1.

2.2 Restructured meat products

In recent years, the alterations of consumer's habits characterized by much agitation and lack of time, as result of the urbanization process and the insertion of women in the labor market have increased the search for food products that are quick and easy to prepare, a factor that is considered important in the decision making of consumers (SALDAÑA *et al*., 2020). In this regard, restructured or comminuted meat products have been a promising alternative whose consumption has been increasing significantly due to their benefits such as convenience in preparation, requirement of few meat trimmings, and for being products with different shapes with good tenderness, juiciness, characteristic flavor and with less cost of acquisition (GADEKAR *et al*., 2015).

The term comminuted meat is used to describe meat, either raw or pre-cooked, which has been cut, shredded, ground or minced into small pieces. Comminuted meat products contain meat and non-meat ingredients like binders and spices. Such products include frankfurters, nuggets, salami, patties, meatballs, bologna-sausages, fermented sausages, among others (BOLGER *et al*., 2017).

Among these products, chicken nuggets stand out for being consumed by all social classes over the world owing to their low production cost, easy and quick preparation, and also

because they meet the sensory expectations of the consuming public, as the lifestyle of modern consumers has changed significantly in the last decades (ASLAM *et al*., 2020; MAGDELAINE; SPIESS; VALCESCHINI, 2008).

According to the Normative Instruction nº. 06 (BRAZIL, 2001), breaded is an industrialized meat product obtained from one or the diverse meat species of slaughtered animals, added to non-meat ingredients, molded or not, and coated with an appropriate coating that characterizes it. The product may be raw, semi-cooked, semi-fried or fried, and its composition may contain fillings. In the sales denomination, the product will be designated as breaded, followed by expressions or denominations that characterize it according to its presentation for sale.

The Normative Instruction nº. 06 (BRAZIL, 2001), about the Technical Regulation of Identity and Quality of Breaded Meat Product, allows the addition of 4% non-meat proteins as aggregated. The requirements regarding the physical-chemical characteristics are 30% of total carbohydrates and a minimum of 10% for protein (10%).

Table 1- Overview summarizing the most recent studies about the incorporation of plant-based source in meat products

Plant-based source	Meat product	Incorporation level	Main results	Reference
Sorghum bran	beef sausages	1-3%	Increased hardness, cohesiveness, resilience, gumminess and chewiness Antimicrobial activity	[1]
Dragon fruit peel powder	Fish nuggets	1-2%	Improved emulsion stability and cooking yield Inhibited lipid oxidation and microbial load	[2]
Amaranth flour	Chicken nugget	0-100%	Decrease hardness, springiness, cohesiveness, gumminess and chewiness Increased all texture-related parameters and cutting force Increased minerals, fiber, fat, protein, and emulsion stability Increased porosity, oil absorption and cooking loss. Decreased 2-Thiobarbituric acid (TBA) values	[3]
Chia (*Salvia hispanica* L.) flour	Chicken nuggets	5-20%	Increased polyunsaturated fatty acids and dietary fiber Decreased the water activity. Oil absorption was not affected Protein, lipid and ash contents, and cooking yield were not affected Addition of up to 10% acceptable by the panelists	[4]
Green peas (*Pisum sativum*)	Chicken nuggets	0-12%	Increased dietary fiber; improved texture-related parameters Produced appealing sensory properties	[5]
Lentinula edodes	Pork sausage	25-100%	Improved moisture, dietary fiber, essential amino acids, and total phenolics content. Increased cooking yield and antioxidant activity. Decreased energy, protein, ash contents, and pH value. Good sensory acceptance achieved at 25% of substitution	[6]
Oyster mushroom waste	Chicken patties	10, 20, and 30%	Effect on hardness, cohesiveness, springiness, and chewiness of the patties Increased hardness and chewines	[7]

[1] - (XIONG *et al*., 2022); [2] - (BISWAS; KANDASAMY; DAS, 2022); [3] - (TAMSEN; SHEKARCHIZADEH; SOLTANIZADEH, 2018); [4] - (BARROS *et al*., 2018); [5] - (ZAINI *et al*., 2021); [6] - (WANG *et al*., 2019); [7] - (WAN-MOHTAR *et al.*, 2020)

Source: Author (2023)

2.3 Plant-based meat analogues (PBMA)

Protein is a vital nutrient for the human body. From 1992 to 2016, global meat consumption increased more than 500% (KATARE *et al*., 2020), and it is expected to increase by 70% with the projected growth in the world's population, which is estimated to reach 9.8 billion people by the year 2050 (CHOUDHURY *et al*., 2020). However, in recent years, it has been increasingly recognized in scientific, political and popular discourses that animal-based food production and consumption have significant, detrimental environmental impacts (NOTARNICOLA *et al*., 2017).

The production and consumption of animal-based foods, generate high amounts of greenhouse gas emissions, use substantial areas of land and aggravate acidification, eutrophication and water scarcity (CLARK *et al*., 2019; SCARBOROUGH *et al*., 2014). At the same time, contemporary dietary guidelines in many European countries recommended reducing red and processed meat consumption (MELTZER *et al*., 2019; REYNOLDS *et al*., 2014). High meat product intake is associated with an increased risk of several chronic disease, such as cancer (BOUVARD *et al*., 2015) and type 2 diabetes (NEVALAINEN; NIVA; VAINIO, 2023).

In order to overcome or reduce the global environmental and health-related concerns of meat production and consumption, meat analogues, or plant-based products that simulate the properties of traditional meat products, have started receiving global attention (BOHRER, 2019). Meat analogue or mimic meat in this context is a foodstuff that seems to be similar in structure to meat but differ significantly in composition. Meat like compound or substance made from plant sources is simply called meat analogue. Plant-based meat, vegetarian meat, meat substitute, mimic meat, meat replacement, synthetic meat, or amalgam meat are some of the most commonly used names. Meat substitute almost has the same aesthetic attributes, such as consistency, taste, physical appearance or chemical based traits of special kind of meat (AHMAD *et al*., 2022).

A global expansion of the PBMA marketplace is occurring along with rapid growth in product offering and availability. The marketplace for meat analogue products in Europe and North America has expanded beyond just vegetarian consumers to now include meat eating and meat loving consumers (BOHRER, 2019). As indicated by Bohrer (2019), the market snapshot of the meat analogue marketplace is projected to grow at a 7.9% compound annual growth rate between the years of 2019 and 2024, with the fastest growing market being the Asia Pacific and

the largest market being Europe. Overall, the global plant-based meat industry is projected to rise from 6 billion in 2018 to $30.9 billion in 2026, with a strong growth momentum and good market potential (SHA; XIONG, 2020).

Apart from the above-mentioned concerns, the shift towards vegetarian or veganism dietary habits encourages the development of PBMA imitating the texture and sensorial characteristics of meat products. The concept of vegetarianism or veganism started to increase in many countries in the mid 20th century as a part of a trend to develop and use meat analogues for the benefit of animals, humans, and environment. Vegetarian and vegan culture is mainly driven by cultural ethical, religious, or environmental issues (ALLÈS *et al*., 2017).

A vegetarian or vegan diet is based on plant foods, including fruits, vegetables, nuts, seeds, whole grains and legumes and their derivates, and excludes the intake of all kind of meat, fish and molluscs and crustaceans. Dairy products, eggs, and honey might be consumed, so that there are various types of vegetarian diets (AGNOLI *et al*., 2017; BALDASSARRE *et al*., 2020). Some studies have highlighted the positive effects of a vegetarian or vegan diet on general health, particularly for avoiding or lowering the risk of obesity and overweight, type 2 diabetes, cardiovascular diseases, and certain types of cancer (MILES *et al*., 2019; PAWLAK, 2017; SALVADOR *et al*., 2019; WEDER *et al*., 2019).

To be successful, PBMA must taste like meat. However, it is difficult, if not impossible, to reconstitute whole muscle meat from PBMA, with respect to the fine texture that microscopically resembles myofilaments for both tenderness and juiciness (SHA; XIONG, 2020). Therefore, the develop of PBMA has largely been limited to restructured products. These formed meat analogues products can be dived into two major groups: coarse-particle products and fine-particle products. The former include PBMA burgers, patties, sausage, meatballs, chicken nuggets and others, while the latter consisted typically of emulsified products such as alternative frankfurters and bologna (SHA; XIONG, 2020). Among these products, legume-based burgers, patties, nuggets, and fried balls, which have been around for many decades and mostly consumed, are undergoing a rapid market expansion (SHA; XIONG, 2020). The most recent studies about the development of PBMA are summarized in Table 2.

Table 2- Overview summarizing the most recent studies about development of plant-based meat analogues (PBMA).

Main ingredients	Incorporation level	Main results	Reference
Banana florest (BF) Raw jack fruit (RJF)	30-60% BF, 30-60% RJF	Similar flavor preference as control High fiber and protein High sensory acceptance large economical potential	[1]
Full fat soy (FFS) Soy protein isolate (SPI) Oyster mushroom (OM)	76-100% FFS, 0-12% OM 76-100% SPI, 0-12% OM	FFS-analog burger had inconstant patterns in the springiness and cohesiveness SPI-analog burger had higher texture-related parameters FFS-analog burger had highest water holding and cooking properties OM improved the overall quality	[2]
Soy protein concentrate (SPC) Wheat gluten (WG) Vegetable oil (VO) Pumpkin powder (PP) Wheat starch (WS) Salt	59% SPC, 30% WG, 5% VO 3% PP, 2.7% WS and 0.3 salt	High degree of texturization, fibrous structure, High hardness and chewiness	[3]
Soy protein concentrate (SPC) Microalgae powder (MP)	30% SPC and 30% MP	Higher vitamins B and E content and tenderness	[4]
Peanut protein powder (PPP) Soy protein isolate (SPI) Wheat gluten (WG)	80% PPP, 10% SPI and 10% WG	Rich fibrous structure, lower hardness Higher springiness	[5]
Soy protein isolate (SPI) Rice protein isolate (RPI)	RPI at 25, 50, 75, and 100%	RPI replacement showed a decreased water absorption, porosity and and specific mechanical energy of the meat analogs; Compared to commercial, RPI and SPI combinations resulted in better nutritional quality	[6]

[1]- (PRIYA *et al*., 2022); [2]- (CHO; RYU, 2022); [3]-(CHIANG *et al*., 2019); [4] (CAPORGNO *et al*., 2020), [5]-(ZHANG *et al*., 2018), [6]- (LEE *et al*., 2022)

Source: Author (2023)

2.3.1 Main challenges about plant-based meat analogues

Undoubtedly, compared to regular meat products, PBMA have a lower negative environmental impact on regulatory and supporting ecosystem services, such as purification of water and air, carbon sequestration, climate regulation, nutrient cycling, and water cycling (CAVENDER-BARES *et al*., 2014; CLUNE; CROSSIN; VERGHESE, 2017; POORE; NEMECEK, 2018; XIE *et al*., 2022). However, sensory properties, nutritional, health, and food safety points of view, can PBMA really be meat substitute? This is also worth to be discussed.

First, from sensory properties point of view, due to natural differences between the muscle and plant-based materials, for instance, structure and size of protein, amino acid profile, peptide sequence, and the chemical composition of both intracellular and extracellular constituents, it is difficult to reproduce the complex and delicate sensory characteristic of animal meat products; the particular challenge is to create the type of highly organized fine texture and water-holding capacity to meat to give PBMA a meat like mouthfeel (SHA; XIONG, 2020).

Another major drawback to the progress of PBMA is the lack of animal meat flavor that consumers are familiar with (GRAÇA; GODINHO; TRUNINGER, 2019). To compensate it, a wide range of spices and herbs, including those used in meat processing, are incorporated to mimic processed meat flavors. However, aftertaste can still be detected in many PBMA. The characteristic beany odor, is thought to be linked with the off flavor products or derivatives, such as hexanal and methanethiol (BOATRIGHT; LU, 2007). Additionally, the lack of distinct red (fresh meat) or pinkish (nitrite-cured meat) meat color is another negative point of PBMA (SHA; XIONG, 2020).

Second, from nutritional point of view, the highly processed nature of most PBMA is not necessarily indicative of good intent. During the cooking process, due to the vigorous processing conditions such as high temperature cooking, PBMA will obviously lose some of the nutrients either naturally present or added as supplements (SHA; XIONG, 2020).

Although it has been claimed that one of the purported advantages of PBMA is improved health benefits, few nutritional studies are available to support the specific health benefits of these materials in comparison with the nutrient profile of meat. For instance, it is not clear whether inorganic minerals in the formulations are of biological efficacy similar to organic heme iron, zinc, selenium, and other types of minerals naturally found in the muscle tissue (SHA; XIONG, 2020). Furthermore, PBMA contain more salt than the meat products they are

formulated to replace, presenting a challenge for reducing sodium level and improving health benefits (SHA; XIONG, 2020).

The lack of clean label is another common negative point observed in PBMA. The huge number of ingredients, commonly is excess of 20 and in some case as many as 40, are found in these products. The additives may include stabilizers, colorants, and preservatives that are usually added in regular meat products, for instance, titanium dioxide, lecithin, and methylcellulose (SHA; XIONG, 2020). Bohrer (2019), in his review study about the formulation and nutritional composition of seven popular commercial PBMA (burgers, nuggets, and hams), reported that each product contained 20 to 30 additives based on the ingredient list. The huge number of additives included, along with saturated fat and high salt content in some of the products, raise the question of whether some PBMA can really be considered as healthier and more nutritious than meat. Moreover, high-temperature cooking of protein foods could produce toxicants and carcinogens, including heterocycle aroma amines (BARZEGAR; KAMANKESH; MOHAMMADI, 2019). This has been reported for meat subjected to high-temperature cooking, such as grilling, frying, baking, and roasting (JIANG; XIONG, 2016; NADEEM *et al*., 2021).

2.4 Cooking methods

Correct cooking is essential because improves the sensory properties of food and makes it more delicious as well as improving the its appearance making it more attractive to consumers by inducing better aroma, color and flavor attributes (ANDRADE *et al*., 2016; TREVISAN *et al*., 2016). Cooking also improves the digestibility and bioavailability of nutrients and makes food safe by decreasing the microbial load, and inactivate anti-nutrient enzymes (DOMÍNGUEZ *et al*., 2014; RASINSKA *et al*., 2019).

However, different cooking techniques as well as cooking conditions, including time and temperature and heating rate may cause significant changes in the physical and chemical properties of food, thus resulting in the decrease of nutritional value due to vitamins and minerals losses (ABDEL-NAEEM; SALLAM; ZAKI, 2021; CLAUSEN; OVESEN, 2005). Moreover, cooking can promote lipid oxidation, the major cause of food quality deterioration. Its effects are reflected in development of off-flavor, texture modification, loss of essential fatty acids or production of toxic and harmful substances, such as nitrosamines, polycyclic aromatic hydrocarbons, and heterocyclic aromatic amines (FLORES *et al*., 2019; NERÍN; AZNAR; CARRIZO, 2016).

Different cooking methods have different effects on product quality because their different heat transfer medium (convection or conduction by contact, air or steam). The effects of thermal processing methods on quality have been extensively explored in different foods (GIANNETTI *et al*., 2021; IBRAHIM *et al*., 2021; LI *et al*., 2019). Various cooking methods, including deep-oil frying, air-frying, oven roasting, and microwaving can be used to prepare food, among which deep-oil frying is the most commonly used method on cooking nuggets.

Deep-oil frying is the most popular cooking method used around the world, due to its unique flavor, texture, and overall taste of the food products (LIBERTY; DEHGHANNYA; NGADI, 2019). It involves simultaneous heat and mass transfer between the food product and surrounding oil; thus, the frying process is complex in nature. In this method heat is transferred from the frying medium to the product surface by convention and from the surface of the core of the food product through conduction (DAS *et al*., 2020). During the frying, the oil and heat transfer to product generates many changes, including protein denaturation, crust formation, loss of moisture, color changes and formation of aromatic compounds, which can be carcinogenic (MIR-BEL; ORIA; SALVADOR, 2012).

Air-frying is a novel technology which can spray hot air around the food product so as to promote the homogenous contact between the food and the mist of oil droplets in hot air (ANDRÉS *et al*., 2013). In this cooking method, the oil usage is substantially low than deep-oil frying, favoring the production of low-fat food (YU *et al*., 2020). Additionally, air frying results in similar flavor and tasty of the common deep-oil fried foods and it has been associated with positive effects to the environment (QIN *et al*., 2022).

Oven roasting often synonymous with baking oven is a widely used cooking method. Oven roasting is achieved by the combination heat transfer mechanisms, conduction from surfaces in direct contact with the food product, convection from hot air, and radiation from heat source. In this cooking method, roasting is generally intense because food is heated at temperatures in 150-300 ^{o}C range. The cooking time can vary based on the desired characteristics of the final product (SRUTHI *et al*., 2021). Similar to deep-oil frying, this method can result in harmful compounds such as acrylamides and others.

Microwave cooking is one of the modern methods that is gaining popularity in household and large-large-scale food applications. Microwave are non-ionizing radiations in electromagnetic spectrum within a frequency band of 300 MHz to 300 GHz (SRUTHI *et al*., 2021). The operation principle of microwave cooking is based on radio waves at 2500 MHz

(SULEMAN *et al*., 2020). When microwaves penetrate into the food matrix, the electromagnetic field interacts with the chemical components of the food and causes the water within the food to heat up due to molecular friction and excitation. Volumetric heating causes the production of vapors in the interior of food, which gets forced to move outside due to pressure gradient allowing rapid removal of moisture from food (PUNATHIL; BASAK, 2016).

The advantages of microwave cooking include selective heating, lower energy consumption, easy process control and penetration to a depth of 5 to 7 cm in food product which results in faster cooking and lower nutrient losses end product in comparison to the conventional heating processes (GUNEL; TORUN; SAHIN-NADEEM, 2020; SULEMAN *et al*., 2020).

2.5 Pequi (*Caryocar brasiliense* Camb.)

Brazilian has over 40,000 different species rich in bioactive compounds, expressing 20% of the world`s flora (CARVALHO; CONTE-JUNIOR, 2021). Despite their enormous potential for agro-industrial processing, a huge number of native fruits are underexploited. Among the native Brazilian fruits, Pequi (*Caryocar brasiliense* Camb.), belonging to family *Caryocaraceae*, popularly known as pequi, piqui, pequiá, stands out due to its nutritional value and unique flavor and peculiar taste, being important for the local communities (CORNELIO-SANTIAGO *et al*., 2022).

This fruit (Figure 1) has a large greenish-brown exocarp, white external mesocarp, internal mesocarp (pulp) composed of yellowish pyrenes and white colored almonds covered with a layer of thorns (GONÇALVES *et al*., 2011). It is also mostly used in cooking in the typical dishes or consumed in the processed forms of creams, liquor, cand, biscuit and jellies, among other products (SILVA *et al*., 2020).

Pequi is a rich source of insoluble fibers, minerals such as zinc, iron, and phosphorus. It is also a good source of unsaturated fatty acids, especially oleic (60.60%) and divers bioactive compounds, including carotenoids, phenolics, tocopherols, and phytosterols (SILVA *et al*., 2022; TORRES *et al*., 2016). These bioactive compounds exhibit various biological properties, such as antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic (BRITO *et al*., 2022; NETO *et al*., 2017; PEREIRA *et al*., 2020; TORRES *et al*., 2016). Thus, pequi fruit can be considered as promising source of functional foods.

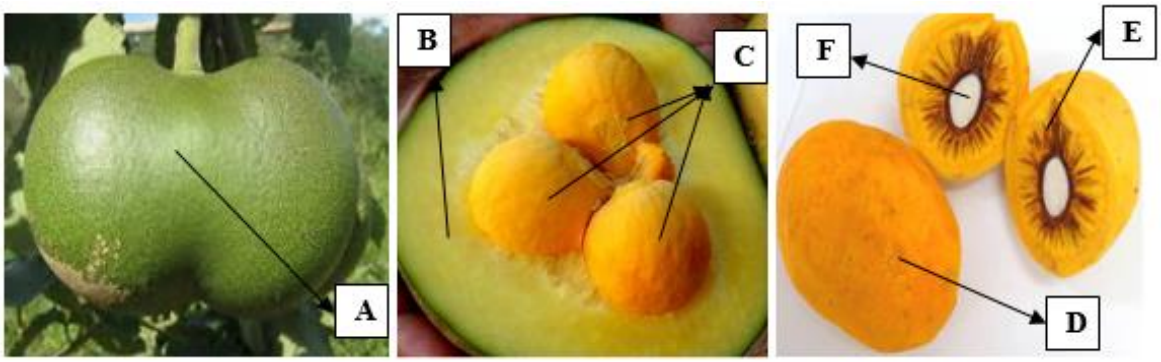

Figure 1. Overview of pequi (Caryocar brasiliense Camb.) fruit. (A) exocarp (B) external mesocarp (C) pyrenes (D) internal mesocarp (E) endocarp (F) almond (seed).

Source: Author (2023)

2.6 Chickpea (*Cicer arietinum* L.)

Chickpea (*Cicer arietinum* L.) is a widely consumed pulse throughout the world, popularly known as a rich source of high-quality protein (20.6-26.7%). Chickpea proteins can be applied in a wide range of food products due to their low cost, well balanced amino acid content, good digestibility and bioavailability than other pulse proteins (BOUKID, 2021; FARIDY *et al*., 2020). The bioavailability of chickpea protein in the human body is higher than in other pulses (KAUR; PRASAD, 2021). Additionally, chickpea proteins have good functional properties, including water holding, solubility, emulsifying, gel and foaming, which make them excellent candidate to be used in the formulation of different food products (SHAABANI *et al*., 2018).

The fat content and fatty acid profile, especially linoleic acid and oleic acid, are higher as compared to other pulses, including pigeon pea, lentil, and moong bean. Chickpea is also rich in dietary fiber, amylose and RS content, which is responsible for the lower glycemic index (KAUR; PRASAD, 2021). Thus, chickpeas are largely incorporated in wide range of food products such as pasta and puffed foods (ALTAF *et al*., 2021). The incorporation of chickpea flour into pasta increased the stickiness (PADALINO *et al.*, 2015) of the pasta and also increased the protein, fat, and mineral and reduced the glycemic index. These studies reported that chickpea flour improved the quality and increased their nutritional value (GOÑI; VALENTÍN-GAMAZO, 2003).

Chickpea also present therapeutic benefits including such as prevention the risk of cardiovascular disease, anti-diabetic, hypo-cholesterolemic, anti-cancerous, and anti-

inflammatory property. Cooking chickpea before consumption results in the elimination of anti-nutritional factors (KAUR; PRASAD, 2021). Considering the protein composition, dietary fiber, and functional properties, chickpea can be considered functional food.

2.7 Sensory analysis

For specific food product to be successful on the market, it needs to be acceptable to consumers. Although instrumental analyses such as texture profile, colorimeters, rheometers can avoid the subjective influence of human factors on the evaluations results from sensory properties of food (MADIETA; SYMONEAUX; MEHINAGIC, 2011), the role of sensory analysis in the food industry for quality control and product development is unquestionable; it can reflect more directly and accurately of perceived intensities of target attributes, such as appearance, color, aroma, taste, and texture (ARES, 2015).

The sensory analysis of food has usually been obtained by performing discriminative and descriptive tests with untrained or trained judges in order to measure specific attribute concerning food quality. In most sensory studies, consumer tests usually focus on hedonic measurements, whereas sensory descriptive methods are normally performed by trained judges (VILLANUEVA; DA SILVA, 2009). Recently, check-all-that-apply (CATA), a rapid descriptive method has been successfully used to evaluate consumers' sensory perception of many different food items (GRASSO *et al*., 2017; JAEGER *et al*., 2020). This method can be used with semi-trained or untrained panelists. CATA presents the participants with a list of terms where they are asked to evaluate the samples according to a liking scale with the aim to identify the key sensory attributes related with the most liked, or disliked, products (VIGNEAU *et al.*, 2022).

CATA has been found to be a reproducible method when asking untrained consumers to utilize it (ARES *et al*., 2014). CATA has also been found to produce comparable product configurations to descriptive. Regularly applied to collect rapid sensory information, CATA questions were also successfully introduced to collect other perceptual measures such as emotional responses (JAEGER *et al*., 2018) or situational appropriateness (JAEGER *et al*., 2019). Different statistical procedures can be later applied to analyze the obtained data. In particular, Correspondence Analysis (CA) is the most used technique to associate the attributes and samples (VIGNEAU *et al*., 2022). This simultaneous representation of both products and CATA attributes, usually onto the first two components, provides a convenient perceptual map summarizing the consumers' sensory description of the products. Besides this factorial

exploratory analysis, univariate analyses such as Cochran's Q test are widely used to test product differences for each CATA attribute (MEYNERS; CASTURA; CARR, 2013)

3 FINAL CONSIDERATIONS

The increase of health-conscious consumers among different socio-economic groups throughout the world, and the rapid population growth associated with shortage of food and protein supply have driven rapid demand for functional meat and plant-based meat analogues products. The incorporation of functional ingredients in meat and meat analogues products have been reported in a large number of literatures, however, very few studies have addressed the use of pequi flour in these novel foods. Therefore, this study could provide valuable information about the potential of pequi flour in the formulation of chicken nuggets and in chickpea meat analogues.

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SECOND SECTION

ARTICLE 1: Nutritional and functional properties, fatty acid composition and volatile profile of pequi pulp flour (*Caryocar brasiliense* Camb)

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Abstract

Brazil native flora is a rich source of underexploited fruit species with high bioactive compounds. This study aimed to develop and characterize pequi pulp flour (PPF) (*Caryocar brasiliense* Camb.) in terms of nutritional value, technological and functional properties to assess its potential as ingredient in food formulation. The results revealed that PPF is a rich source of dietary fiber (11.73g/100g), lipids (66.40 g/100g), carotenoids (558.67 µg/100g), energy value (658.95 kcal/100g), K (139.05mg/100g), Mg (53.03 mg/100g), oleic acid (56.59%) and palmitic acid (36.22%). Additionally, PPF showed good technological properties, such as water holding capacity (2.98 g/g), oil holding capacity (1.20 g/g), water solubility index (15.18 g/100g) and swelling capacity (8.57 mL/g) and low levels of Ca (0.71 mg/100g), Mn (1.26 mg/100g), Zn (1.88 mg/100g), Fe (0.96 mg/100g), Cu (0.91 mg/100g) and B (0.91 mg/100g). The phenolic compounds were 143.16 mg GAE/100g and antioxidant activity by ABTS (11.36 µM trolox/g), DPPH (7.40 µM trolox/g), and FRAP (12.13 µM trolox/g). A total of 40 volatile compounds detected in PPF; the most dominant compounds were esters (50.02%), followed by terpenes (25.98%) and acids (18.04%). The results suggested that PPF can be used as functional ingredient.

Keywords: Dietary fiber, bioactive compounds, functional food, technological properties, carotenoids.

1. Introduction

In recent years, the demand for fruits and vegetables has been considerably increased due to an increase in the global population, changing dietary habits, and consumer perception of the health benefits offered with such important food commodities (Zia et al., 2021). Additionally, the current scenario of COVID-19 pandemic, which has turned into global crisis and will result in possible irreversible effects, has also led to a major acceleration in this demand and consumption (De Backer et al., 2020; Poelman et al., 2021).

Brazil has a wide variety of species of native and exotic fruits with worldwide potential for consumption and industrialization (da Silva et al., 2022; Guedes et al., 2021). Most of these native fruits stand out due to their peculiar taste, exotic flavor, high nutritional value and bioactive compounds, which are related to human health benefits. However, due to the lack of scientific knowledge, inefficient governmental support and lack of cooperation with emerging biodiversity markets, these fruits are poorly exploited being only grown for consumption in local dietary (Miranda et al., 2021; Ribeiro & Soares Filho, 2022)

Among the native fruits, pequi (*Caryocar brasiliense* Camb.), also known as piqui or piquiá, is a native fruit from Brazilian Cerrado highly appreciated by local communities, owing its exotic and unique flavor, as an ingredient in regional dishes such as rice, beans, and pork (Leão et al., 2017; Silva & Fonseca, 2016). Data from Brazilian Institute of Geography and Statistics - IBGE, indicated that the production of pequi fruits was approximately 63,520 tons in 2020, with large contribution to the north (~ 25,232 tons) and southeast (~ 32,928 tons) regions (IBGE, 2020).

Currently, pequi is getting a lot of attention from researchers and consumers due to its high nutritional value, pleasant color, flavor and the presence of bioactive compounds (Pinto et al., 2022; Santos et al., 2022). Pequi is rich in vitamins, minerals, insoluble fibers, bioactive compounds, such as phenolics, tocopherols, phytosterols, carotenoids, especially beta-carotene, in addition to unsaturated fatty acids, being oleic acid (60%) the most dominant (Pinto et al., 2022; Silva et al., 2022; Torres et al., 2016). The bioactive present in pequi have been linked with health beneficial effects include anticarcinogenic, anti-oxidant and anti-inflammatory, analgesic, antibacterial, and healing an gastroprotective (Brito et al., 2022; de Lacerda Neto et al., 2017; Pereira et al., 2020; Torres et al., 2016). Therefore, pequi has a great potential to be incorporated in the food system as functional.

However, pequi in its fresh from is highly perishable with very short-life during storage because of high moisture content associated with microbial growth and infections. Thus, improvement of its shelf-life is a serious concern for its proper exploitation without generation of huge amount of fruit-waste (Ghosh & Singh, 2022). Among various methods employed in extension of fruits shelf-life, processing of pequi pulp in flour is a good alternative way to add value and enlarge its use as functional ingredient in different food formulations.

Therefore, the objective of this study was to develop and characterize pequi pulp flour (PPF) in terms of proximate composition, bioactive compounds, fatty acids composition, volatile compounds and technological properties in order to evaluate its potential to be used as functional ingredient in food formulations.

2. Material and methods

2.1. Materials

Frozen pequi fruit (*Caryocar brasiliense*) without the exocarp (a greenish-brown outer skin) and the white external mesocarp, harvested in 2021, were obtained from two Fruit Pulp Industries-Barra do Garças (Mato Grosso State, Brazil) and Goiânia (Goias State, Brazil). The fruits were then transported to the Pilot Plant in the Laboratory of Post-Harvest of Fruit and Vegetables Fruit of Federal University of Lavras (UFLA, Lavras, Brazil), where the experiment was carried out.

2.2. Preparation of pequi pulp flour (PPF)

Fruits consisted of the internal mesocarp (yellowish-orange pulp) and seeds, without any physical injury and uniform in size and color were visually selected, sanitized with sodium hypochlorite solution (100 mg/L) for 15 min and then rinsed in distilled water to remove the residual chlorine. Afterward, they were bleached in a water bath at 80 ^{o}C for 8 min. After 10 min cooling over in an ice bath, the fruits were then manually pulped using the stainless knife and the pulp and seeds were removed. Subsequently, the pulp was dried in an oven with forced air circulation (Solab SL 102 model, Piracicaba, São Paulo, Brazil) at 40 ^{o}C for 30 h. The dried pulp was first crushed using an industrial blender (Poli model LS-06, Brusque/Brazil), and then in a knife mill (Tecnal T650 model, Piracicaba, Brazil) and passed through a 20 – mesh sieve (0,85mm) to obtain the PPF. The resulted flour was vacuum-packed in polyethylene plastic bags containing approximately 500g and stored frozen at -20 ^{o}C until further use.

2.3. Proximate composition

The proximate composition analysis was carried out in triplicate. The moisture, protein, ash and total dietary fiber were performed according to previous published methodologies (AOAC, 2019). For total moisture content (AOAC 950.46B), 10 g of the sample were dried to constant weight at 105 ^{o}C. Protein content (AOAC 981.10) was determined by the Kjeldahl method and the factor 6.25 was applied for conversion of nitrogen to crude protein. The ashes were analyzed by combustion at a temperature of 550 ^{o}C (AOAC 920.153). The fat content was analyzed by the cold extraction (chloroform/methanol/water) according to Bligh & Dyer (1959). The total dietary fiber content was analyzed by the enzymatic-gravimetric method (AOAC 991.43) using a Total Dietary Fiber Assay Kit. The carbohydrate content was determined by subtracting the sum of moisture, protein, fat, ash, and total dietary fiber from 100%. Energy was calculated using the Atwater conversion factors (4 for carbohydrate and protein and 9 kcal for fat). The outcomes were express in fresh matter.

2.4. Mineral profile

The mineral profile was determined using the method implemented by Malavolta et al. (1997). First, the samples were subjected to nitroperchloric digestion (NPD). The NPD extracts were then used for Analysis of mineral profile. The P content was assessed by colorimetry, K content by flame photometry and Ca, Mg, Mn, Zn, B, Cu, Zn, and Fe content by atomic absorption spectrometry SpectrAA 110 model (Varian Inc., Palo Alto, CA, USA).

2.5. Extraction and determination of total carotenoids

The total carotenoids of PPF were extracted and quantified by using the method of Rodriguez-Amaya (2001) with minor modifications. Briefly, 2.5g of the of frozen PPF samples were mixed with 20 mL of cold acetone and vigorous shaking on a shaker (Nova ethics model 109-2TCM, Vargem Grande Paulista, Brazil) for 20 min. The residue was separated from the liquid phase by filtration using a qualitative filter paper with 14-μm porosity and washed three times with acetone (20 mL). The filtrate was transferred into a 250 mL separatory funnel to which 30 mL of petroleum ether and 100 mL of water were added. After phase separation, the lower phase containing acetone and water was discarded and the washing process was repeated three time. The resulting total petroleum ether layer was filtered using a filter paper with 14-μm porosity and the volume was completed to 50 mL with petroleum ether. The absorbances were measured a UV-VIS spectrophotometer at 444, 450, 456, 462, and 470 nm for α-carotene,

β-carotene, δ-carotene, γ-carotene, and lycopene, respectively. The results were expressed in μg/100g of the total carotenoids content using the following equation (1).

$$C = \frac{OD \times V \times 10^6}{A^{1cm1\%} \times W} \times 100 \quad (1)$$

Where, C is the total carotenoid content (μg/100g); OD is the absorbance at each specific wavelength (444, 450, 456, 462, and 470nm); V is the total volume of sample extract solution (mL); $A^{1cm1\%}$ is the specific absorption coefficient of particular carotenoids (α-carotene, β-carotene, δ-carotene, γ-carotene, and lycopene); W is the weight of the sample (g).

2.6. Extraction of phenolic compounds

Extracts for phenolic compounds and antioxidant activity were obtained by the method implemented by An et al. (2022), with few modifications. Briefly, 2.5 g of frozen PPF were mixed with 10 mL of 80% (v/v) methanol and subjected to ultrasonic bath at 4◦C for 30min. The mixture was then centrifuged at 4000 x g for 15 min and the supernatant was collected. The procedure was replicated twice and the supernatants were combined and filtered through filter paper with 14 μm porosity and stored at − 80 ◦C prior to analysis.

2.7. Determination of total phenolic content (TPC)

TPC were determined by the Folin-Ciocalteau method as described by Zitha et al. (2022). An aliquot of 30 μL of extract was mixed with 150 μL of Folin-Ciocalteu reagent (10%, v/v) and 120 μL of sodium carbonate (4%, w/v) in a 96-well microplate. After incubation in darkness for 2 h the absorbances were measured at 720 nm using a microplate reader (Biochrom EZ Read 2000). The results were estimated as milligram gallic acid equivalents per 100 g of fresh weight of the sample (mg GAE/100 g FM).

2.8. Determination of antioxidant activity

Antioxidant activity by DPPH assay was carried out following the method implemented by Rufino et al. (2007) with small changes. In brief, 3 μL of extract, 27 μL of ethanol and 270 μL of DPPH• solution in ethanol (0.0236 mg/ mL) were mixed in a 96-well microplate. After 30 min incubation in the dark at room temperature the absorbance was assessed at 517 nm using a microplate reader (Biochrom EZ Read 2000). Antioxidant activity was determined from a

five-point standard Trolox curve (100–2000 µm/L in ethanol) and the results were reported as µmol Trolox equivalent (TE)/g extract.

Antioxidant activity by ABTS assay was performed as described by Ilyasoğlu et al. (2015) with small changes. The ABTS◦+ stock solution was generated by reacting 5 mL of ABTS◦+ solution (7 mM) with 88 µL of K2S2O8 solution (2.45 mM) for 16 h at room temperature in darkness. Prior to analysis, the resulted stock solution (ABTS◦+) was diluted with ethanol to an absorbance of 0.70 ± 0.05 at 734 nm. Then, an aliquot of 10 µL of the extract was mixed with 290 µL of ABTS◦+ diluted solution and the absorbance was taken at 720 nm after 6 min, using a microplate reader (Biochrom EZ Read 2000). The results were given as µmol Trolox equivalent (TE)/g extract.

Antioxidant activity by ferric reducing antioxidant power (FRAP) assay was as described by Rufino et al. (2006), with minor changes. FRAP reagent was prepared by mixing 40 mM TPTZ (diluted in 40 mM HCl), 300 mM of acetate buffer (pH 3.6), and 20 mM of FeCl3 in a ratio of 10:1:1 (v/v/v). 3 µL of the extract was mixed with 270 µL of FRAP reagent and 27 µL distilled water in a 96-well microplate, and the mixture was incubated at 37 ◦C for 30 min. The absorbance was measured at 595 nm using a microplate reader (Biochrom EZ Read 2000). The antioxidant activity of the samples was determined from a five-point standard Trolox curve (100–2000 µm/L in ethanol) and the results were reported as µmol Trolox equivalent (TE)/g extract.

2.9. Technological properties

The color parameters luminosity (L*), chroma (C*) and hue angle (h) of PPF were measured using a Minolta CR-400 (Konica Minolta Sensing, Inc., Osaka, Japan) colorimeter, operating with D65 illuminant, 8 mm aperture, and 10^{o} observer angle. The measurements were recorded at 10 points.

Water and oil holding capacities (WHC and OHC), water solubility index (WSI) and swelling capacity (SC) were assessed as described by Wang et al. (2015), with small changes. Briefly, 2g of sample were placed in a tube and mixed with 20 mL water or soy oil separately, shaken in a vortex and centrifuged at 4000 x g for 15 min. The supernatant contains oil was discarded and the supernatant with water was reserved for WSI determination. The mass of residue remained after centrifugation was measured and divided by the initial mass of the samples. The reserved supernatant from WRC determination was dehydrated a 105^{o}C for 12h

and WSI measured as the percentage mass ration between the dehydrated and original samples. Finally, for swelling capacity determination, 150 mg of samples were mixed with water, shaken, followed by decantation for 12h; the final volume (mL) occupied by the sample was recorded and reported in mL/g of sample.

2.10. Fatty acids profile

The total fat extraction was based on the procedure describer by Bligh & Dyer (1959). Briefly, 4g of PPF were extracted using methanol, chloroform and water (1:1:2:0.8, v/v/v) mixture and determined by gravimetry in an oven with forced air circulation (Solab SL 102 model, Piracicaba, São Paulo, Brazil) at 105 ^{o}C. Then, 20 mg of fatty acid were converted to fatty acid methyl esters (FAME) by the derivatization reaction proposed by Hartman & Lago (1973). The lipid fraction was added 1mL of methanolic potassium hydroxide solution (0.4 M) and the whole was subjected to a boiling point water bath for 10 min. After cooling, 3 mL of methanolic sulfuric acid solution (1 M) was added and again subjected to heating for 10 min. Finally, 2 mL of hexane were added to solubilize the FAME. A fused silica capillary column

The hexane-diluted FAMEs were quantified by gas chromatography (Shimadzu GC 2010) equipped with a fused silica capillary column (Supelco SP-2560, Bellefonte, PA, EUA; 100 m x 0.25 mm x 0.25 µm film thickness), flame ionization detector and injector split ratio was 1:50. The injector and detector temperatures were 260 ^{o}C. Helium was used as the carrier at a constant flow of 0.8mL/min. The operating column conditions were the following: initial temperature of 140°C/5 min, which increased to 240 ^{o}C at a rate of 4°C/min and it remained at this temperature for 30 min for a total run time of 60 min. The fatty acids peaks were identified by comparison with standards available FAME mixtures (37-component FAME Mix; Supelco Inc., Bellefonte, PA, USA) and the results were expressed as percentage of the total detected FA methyl esters (FAME). The atherogenicity index (AI) and thrombogenicity index (TI) were determined according to equations (3) and (4), developed by (Ulbricht & Southgate, 1991)

$$AI = (C12{:}0 + 4 \times C14{:}0 + C16{:}0) / \left(\sum MUFA + \sum n\text{-}6 + \sum n\text{-}3\right) \quad (3)$$

$$TI = (C14{:}0 + C16{:}0 + C18{:}0) / \left[\left(0.5 \times \sum MUFA\right) + \left(0.5 \times \sum n\text{-}6\right) + \left(3 \times \sum n\text{-}3\right) + \left(\sum n\text{-}3 / \sum n\text{-}6\right)\right] \quad (4)$$

2.11. Volatile compounds analysis

Volatile compounds profile of the pequi was analyzed by the headspace-solid phase microextraction (HS-SPME) technique described by Gomide et al. (2022), with minor adjustments. 5 g of PPF were placed in a 10 mL sealed glass vial with PTFE-silicone septum (Supelco, Bellefonte, PA, USA). Divinylbenzene/Carboxen/Polydimethylsiloxane (50-30µm coating thickness; 1 cm length; Supelco, Bellefonte, PA, USA) fiber was used for the extraction of volatile compounds. Prior to extraction, the fiber was conditioned at 220 ºC for 5 min according to manufacturer instructions. The samples were incubated at 40 ºC for 10 min and then extracted at 40 ºC for 30 min, with a constant desorption of 2 min. After extraction, the volatile compounds were separated and identified using a GC-MS QP2010 plus (Shimadzu Corporation, Japan) equipped with an AOC-5000 automatic injector for liquids and gases (Shimadzu, Japan) and an SLBTM column (30 m x 0.25 mm x 0.25 µm 5% phenyl, 95% dimethylsiloxane film. The CG conditions were as follows: the injector temperature was maintained at 220 ºC under splitless mode; high purity helium (99.999%) was used as carrier gas, and the flow rate was kept constant at 1.0 mL/min. The initial oven temperature was set at 40 ºC for 4 min, then increased to 80 ºC at a rate of 2 ºC/min. After, the temperature was increased from 80 ºC to 140 ºC at a rate of 4 ºC/min, and finally increased further for 200 ºC at a rate of 8 ºC/min, where it was maintained until the end. The mass spectrometer was operated under electron impact mode (70 eV) with a full scan mode from 45 to 60 Da and solvent cut-off in 0.55 min. The interface and ion source temperatures were 240 ºC and 200 ºC, respectively. Volatile compounds identification was made by comparing their mass spectra with those of the database (NIST 8, Willey 8 and FFNSC 12) and comparing the retention indices (RI) obtained from the retention time (RT) of homologous series of n-alkanes (C8-C20) with the available literature data. The volatile compounds were reported as relative peak areas (peak area of each compound/total area) x 100.

2.12. Statistical analysis

Descriptive data were expressed as means ± standard deviation (SD) of five replications in triplicate and the results were assessed by R version 4.0.4 (R Core Team, 2020).

3. Results and discussion

3.1. Proximate composition

The results of proximate composition of the pequi pulp flour (PPF) are summarized in Table 1. The moisture content was below the acceptable value (9g/100g) for conservation of powders (Soquetta et al., 2016) and was within the recommended value for vegetables flour, according to the Brazilian legislation (< 15g/100g) (ANVISA, 2005); however, the value reported in the present study was higher than that reported by Silva et al. (2022) (4.2g/100g) in pequi pulp dried under temperature conditions of 60, 70, and 80ºC, but similar to that observed by Aquino et al. (2009), who found moisture value of 7.41g/100g in dehydrated pequi at 60ºC for 4h. These differences might be related to drying conditions.

The lipids content of PPF was similar to that reported in Brazil nut, one of the most important sources of lipids, with approximately 68.60g/100g of lipids (Medeiros et al., 2010) and higher than that found by Silva et al. (2022), who reported lipids content of 62.63g/100g. The lipids content value found in this study was high in comparison to other oil species from Brazilian Cerrado such as cupuaçu (24.4g/100g), tucumã (40.49g/100g), pupunha (8.22g/100g), pracaxi (53.42g/100g), buriti (20g/100g) and açaí (24.75g/100g) (Bataglion et al., 2020; Cedrim et al., 2018; Costa et al., 2020; Ibiapina et al., 2022; Teixeira et al., 2018). Therefore, considering the highest lipid content obtained in the present work, PPF can be incorporated in the diet of local population as a potential alternative of low-cost lipid with high nutritional and energy.

The protein content reported in this work was low than that found in PPF by Silva et al. (2022), who found value of 5.0g/100g and higher than that reported in other fruits of Cerrado biome, including mangaba (1.20g/100g), feijoa (1.42g/100g), cajuí (1.10g/100g), jatobá (1.70g/100g) and cagaita (2.5g/100g) (Almeida et al., 2020; de Lima et al., 2015; Rocha & Figueiredo, 2013).

The contents of ash were similar to ones reported by Silva et al. (2022) in PPF (2.10g/100g) and by Rojas-Garbanzo et al. (2012) in palm flour (1.78g/100g), but were low than that found by Santos et al. (2018) in tucumã fruit (2.58g/100g) and by Costa et al. (2020) cupuaçu (5.2g/100g).

PPF can be considered as a rich source of dietary fiber, since the content of TDF obtained in the present study was above the minimum recommended (6.0g/100g), according to

Brazilian legislation (ANVISA, 2005). This finding was similar to the one reported by Silva et al. (2022) for PPF. Dietary fiber is known to have several positive health effects. For instance, recent works have demonstrated that high intake of dietary fiber-rich food reduced by 15-30% all cause and cardiovascular diseases, type 2 diabetes, colorectal cancer risks (Reynolds et al., 2019).

The content of the available carbohydrates (excluding the fiber fraction) found was similar with that found in Brazil nut (6.56g/100g) and low that that reported by Silva et al. (2022) when working with PPF dehydrated under different temperatures (60, 70, and 80^{o}C). PPF is a good source of energy; the energy value was similar to that found by Silva et al. (2022) for pequi flour (688.35 kcal) and higher than the contents reported in açaí flours (489.39kcal) and amaranth (347.61 kcal) (Menezes et al., 2008; Silva et al., 2018). The high energy value is related to the high its high lipid content (66.40g/100g).

The mineral profile results revealed that K, followed by Mg and P were the highest macrominerals, whereas Zn and Mn were the microminerals found in high concentrations. Among the most predominant minerals in pequi pulp flour, Fe, K, Mn have been highlighted due to their positive effects in prevention of some diseases such as iron deficiency anemia and hypokalemia (Santos et al., 2018). Moreover, microminerals such as Zn and Fe are cofactor of various enzymes (Mallek-Ayadi et al., 2022).

3.2. Technological properties

Color parameters (L*, C* and h), WHC, WSC, SC and OHC of PPF are depicted in Table 1. The L* value showed a high luminosity in comparison with flours from other fruits such as feijoa peels flours (61.5 and 66) and buriti by-products flours (ranged from 53.13 to 62.38) and low than orange-fleshed sweet potato (Pereira et al., 2019a; Resende et al., 2019). Resende et al. (2019) stated that lighter flours are important due to their possible to be used in wide range of products and in high amount without compromising the natural color of food.

The hue angle (h) value was in range between orange and yellowish hue and high than that found in buriti by-products flours (ranged from 61.94 to 68.09) (Resende et al., 2019). This might be related to the presence of antioxidant compounds, such as carotenoids, which provides the golden yellowish color of pequi fruit. The color intensity (c*) was also higher than reported in buriti by-products flours (23.51-32.22) (Resende et al., 2019).

Water holding capacity (WHC) and water solubility capacity (WSI) are technological properties commonly used to characterize the hydration capacity of fiber (Lundberg et al., 2014). WSI reflects the quantity of water-soluble matter in a food. The WHC value was high when compared with that found in defatted rice bran flour (1.9g/g), and in fruit residues including buriti (1.10-1.6g/g), lemon (1.8g/g), apple (1.9g/g) and orange (1.6g/g) (Almeida et al., 2020; Resende et al., 2019; Wang et al., 2016).

The WSI was in range of that obtained from buriti by-products (ranged from 4.46 to 23.61g/100g) and low compared to that observed in pequi by-products (16.6 and 19.84g/100g) (Leão et al., 2017; Resende et al., 2019). Oil holding capacity (OHC) is one of the most important technological properties mostly used to investigate the ability of fiber to adsorb lipophilic components (Jiang et al., 2022). The OHC value for PPF was similar to that from pequi peels (1.23-1.35g/g) and buriti by-products (1.18-1.27g/g) (Leão et al., 2017; Resende et al., 2019), however, low than that reported in mango peel (2.7g/g) (Larrauri et al., 1996).

Swelling capacity (SC) indicates how much the fiber matrix swells when water is absorbed in the product (López-Vargas et al., 2013). The SC value reported in this study was higher than those found for orange peel (4.83g/g) (Wang et al., 2015) and buriti by-products (ranging from 3.70 to 11.36 mL water/g) (Resende et al., 2019), but low than found in coconut (20mL water/g) (Raghavendra et al., 2006).

3.3. Total carotenoids, total phenolic content (TPC) and antioxidant activity

The results of total carotenoids content, TPC, antioxidant activities are displayed in Table 1. PPF is a good source of carotenoids, however the total carotenoids content found in this study was inferior to that reported by Silva et al. (2022) of 307.05mg/100g in dried PPF. Carotenoids are one of the most important colored pigments commonly found in food are considered to have a functional role against several diseases (Mehmood & Zeb, 2020).

Regarding the TPC, the PPF exhibited higher levels than those reported in pequi flour with the epicarp (17.4g/100g) (Leão et al., 2017) and in giant pequi pulp (101.42 mg GAE/100g) (da Silva et al., 2020) . However, the amount of TPC was low than okara flour (210 mg/GAE), buriti pulp (435.08 mg GAE/100g) (Resende et al., 2019), feijoa peel flours (500 and 640 mg GAE/100g) (Almeida et al., 2020) and mangaba (169 mg GAE/100g) (Rufino et al., 2010). The concentration of phenolic compounds in fruits depend on various factors including maturation stage, environmental factors, extraction methods, difference in varieties,

season and the effects of treatments (Majerska et al., 2019). Phenolic compounds have been linked with a huge variety of biological properties, such as antioxidant, anti-inflammatory and antitumoral activities, which a beneficial to human health (Canaan et al., 2022).

The antioxidant activity of PPF obtained by the DPPH assay was higher when compared with passion by-products (1.5 μmol TE/g) and low than that found in mango by-product (31.7 μmol TE/g). For the FRAP assay the result was superior to that of passion fruit by-product (2.5 μmol TE/g) and similar to mango fruit (13.7 μmol TE/g) (Martínez et al., 2012). Result based on ABTS reagent was higher than reported for some exotic fruit co-products such as passion fruit (5.5 μmol TE/g) and pineapple (7.7 μmol TE/g) (Martínez et al., 2012). Overall, the findings indicated that PPF is a good source of TPC, total carotenoids and antioxidant potential, demonstrating that can be incorporated in food matrices to develop functional food.

Table 1. Proximate and mineral composition, technological properties, total phenolic content, antioxidant activity and carotenoids of pequi pulp flour (PPF).

Analysis	PPF*
Proximate composition	
Moisture (g/100g)	6.72 ± 0.16
Lipidis (g/100g)	66.40 ± 3.27
Proteins (g/100g)	3.76 ± 0.32
Ash (g/100g)	1.82 ± 0.28
TDF (g/100g)	11.73 ± 2.42
Carbohydrates (g/100g)	7.94 ± 0.18
Energy value (Kcal/100g)	658.95 ± 22.96
Mineral composition	
P (mg/100g)	25.23 ± 0.12
K (mg/100g)	139.05 ± 3.83
Ca (mg/100g)	0.71 ± 0.10
Mg (mg/100g)	53.03 ± 2.06
Mn (mg/100g)	1.26 ± 0.52
Zn (mg/100g)	1.88 ± 0.15
B(mg/100g)	0.91 ± 0.10
Fe (mg/100g)	0.96 ± 0.01
Cu (mg/100g)	0.91 ± 0.10
Technological properties	
L*	69.48 ± 1.56
C*	50.33 ± 3.30
h	76.90 ± 0.64
WHC (g /g)	2.98 ± 0.16
WSI (g/100 g)	15.18 ± 0.99
SC (ml/g)	8.57 ± 0.86
OHC (g /g)	1.20 ± 0.06

Total phenolic content, antioxidant activity and carotenoids	
TPC (mg GAE/100g)	143.16 ± 4.27
ABTS (µM trolox/g)	11.36 ± 0.71
DPPH (µM trolox/g)	7.40 ± 0.87
FRAP (µM trolox/g)	12.13 ± 1.27
Carotenoids (µg/100g)	558.67 ± 22.51

* Data were expressed as means ± standard deviation (SD); TDF: total dietary fiber; WHC: water holding capacity; OHC: oil holding capacity; WSI: water solubility index; and SC: swelling capacity.

3.4. Fatty acid profile

Fatty contents and composition in PPF are presented in Fig. 2. A total of 14 fatty acids were identified, including 7 types of saturated fatty acids (SFAs), 4 types of monounsaturated fatty acids (MUFAs), and 3 types of polyunsaturated fatty acids (PUFAs). MUFAs (57.45%) were the dominant fraction, followed by SFAs (39.83%), and PUFA (2.72%) with a relatively lower concentration. Palmitic (C16:0) and stearic acids (C18:0) of SFAs, oleic acid (C18:1n9c) of MUFAs, and linoleic acid (C18:2n6c)) of PUFAs were the main fatty acids in PPF. These results are in accordance with those observed by other authors (da Silva et al., 2020; Nascimento-Silva & Naves, 2019; Torres et al., 2016) who reported that MUFAs and SFAs were the dominant group of fatty acids in pequi pulp.

The oleic acid content found in this study is similar to that reported by Lorenzo et al., (2021) in previous work with pequi oil and higher than that reported in some oil species from Amazonian fruits such as pracaxi and cupuaçu (47.3% and 41.6%, respectively) (Bezerra et al., 2017; Serra et al., 2019). However, the oleic acid content is low in comparison to buriti oil (Lima et al., 2017). It has been reported that consumption of food rich in MUFA, particularly oleic acid had beneficial effects on metabolic syndrome and cardiovascular health (Bergouignan et al., 2009; Serra-Majem et al., 2006). Moreover, a wide prospective cohort study concluded that diet rich in oleic acid was inversely associated with ulcerative colitis development, with huge effects sizes in a dose-dependent manner.

The PUFAs/SFAs ratio was below the recommended ratio (> 0.4), indicating that pequi flour is poor source of PUFA (Wood et al., 2008). The n-6/n-3 ratio is considered one of the most important indices to assess the lipid quality and it can help to prevent the emergence of several diseases (Abdel-Naeem et al., 2021). The n-6/n-3 ratio found in the present study was between the recommended ratio (2.5-8:1) for good fat quality of high-rich fat products (WHO/FAO, 2003).

The atherogenic index (AI) and thrombogenic index (TI) are crucial tools mostly used for estimating the chance of food to cause coronary heart diseases; they also used to assess the nutritional quality of lipids, and the acceptable limit of these indices for a good nutritional quality of lipids is < 1.0 (Weber et al., 2008). The results reported in this work were low in comparison with those reported by da Silva et al. (2020) (AI=1.92 and TI=1.88) in new population of pequi and higher than those of some oil species from Amazonia (AI=0.10-1.85; and TI=0.18-0.70) (Pereira et al., 2019b). However, the AI value observed in this work was within the acceptable limit (< 1.0), whereas the value of TI was quite higher. It has been reported that the low values of these indices have positive effects in lowering the incidence of developing coronary heart diseases (Ulbricht & Southgate, 1991).

Table 2. Fatty acid composition of pequi pulp flour (PPF)

Fatty acid (%)	PPF
Butyric Acid (C4:0)	0.63 ± 0.07
Lauric Acid (C12:0)	0.08 ± 0.01
Myristic Acid (C14:0)	0.25 ± 0.01
Palmitic Acid (C16:0)	36.22 ± 0.14
Heptadecanoic Acid (C17:0)	0.07 ± 0.00
Stearic Acid (C18:0)	2.38 ± 0.01
Arachidic Acid (C20:0)	0.18 ± 0.00
Palmitoleic Acid (C16:1)	0.60 ± 0.00
cis-10-Heptadecenoic Acid (C17:1)	0.07 ± 0.00
Oleic Acid (C18:1n9c)	56.59 ± 0.10
cis-11-Eicosenoic Acid (C20:1)	0.19 ± 0.00
Linoleic Acid (C18:2n6c)	2.14 ± 0.00
Linolenic Acid (C18:3n3)	0.48 ± 0.00
cis-13,16 - Docosadienoic Acid (C22:2)	0.10 ± 0.00
ΣSFA	39.83 ± 0.09
ΣMUFA	57.45 ± 0.10
ΣPUFA	2.72 ± 0.05
ΣPUFA/ΣSFA	0.07 ± 0.00
Σ n-3	0.48 ± 0.00
Σ n-6	2.14 ± 0.04
Σn-6/Σn-3	4.42 ± 0.04
AI	0.62 ± 0.00
TI	1.23 ± 0.01

* Data were expressed as means ± standard deviation (SD); SFA: saturated fatty acids, MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; AI: atherogenic index and TI: thrombogenic index.

3.5. Volatile compounds (VCs) profile

Flavor is one of the most important sensory attributes that affects the quality perception of food and consumer preference (Wibowo et al., 2019). A total of 40 VCs, including esters (20), terpenes (6), acids (4), alcohols (2), ketones (2), pyrazines (2), and others (4) were detected (Table 3). Among them, esters (50.02%) were the most dominant, followed by terpenes (25.98%) and acids (18.04%), which were the main VCs in pequi flour. These results are in accordance with the data observed by other authors (Belo et al., 2013; da Silva et al., 2020; Damiani et al., 2009; Maia et al., 2008) who found that esters and terpenes were the key VCs that substantially contribute to the overall pequi fruit aroma.

Esters are important VCs with low-odor thresholds, which possess typical flavor related with some specific fruits (Chen et al., 2020). Among esters, ethyl hexanoate (32.22%), ethyl octanoate (9.96%) and 2-hydroxy-propanoic acid ethyl ester (4.58%) were found to be the predominant characteristic aroma in PPF. Ethyl hexanoate has been reported as a VC that provides the fruity aroma in various fruits such as pear, pineapple, strawberry, and mango (Zhu et al., 2020). Ethyl octanoate was also reported as the dominant ester VC in fruit pulp from *annona crassiflora* and contributes to the floral and fruity sweet flavor (Carvalho et al., 2022).

Terpenes constitute the major class of plant volatile and substantially contribute to floral and fruits notes of a wide variety of food (Chen et al., 2020; Pott et al., 2019). They can be generated in several scenarios during fruit ripening, such as plant reproduction as well as in defensive mechanism against herbivores and pathogens. Among the terpenes found in this study, (E)-β-Ocimene (16.83%), limonene (5.04%) and (Z)-β-Ocimene (3.31%) were detected in high concentrations. These compounds have also been reported to play a crucial role in andaliman (*Zanthoxylum acanthopodium* DC) sensory acceptability due to high correlation to favorable aroma attributes (citrus, orange peel, acidic) (Suharta et al., 2021). In addition, limonene is reported to reduce tumor growth, induce apoptosis, among others (El Bakali et al., 2022).

Table 3. Relative contents (%) of volatile compounds in pequi pulp flour (PPF)

Number	Volatile compound	RI	PPF*
	Esters		
1	2-hydroxy-propanoic acid ethyl ester	787	4.58 ± 0.60
2	Ethyl butyrate	800	0.14 ± 0.01
3	2-Methoxyethyl acetate	816	0.11 ± 0.01
4	Ethyl isovalerate	848	0.45 ± 0.01
5	Isopropyl 3-methylbutanoate	891	0.10 ± 0.01
6	Methyl hexanoate	921	0.87 ± 0.03
7	Ethyl 3-hydroxybutyrate	932	0.06 ± 0.00
8	3-Methyl-4-octanone	939	0.53 ± 0.02
9	Ethyl hexanoate	996	32.22 ± 0.60
10	Isoamyl butylate	1004	0.21 ± 0.01
11	Ethyl E-2-hexenoate	1042	0.56 ± 0.02
12	Isoamyl butyrate	1053	0.81 ± 0.02
13	Ethyl heptanoate	1094	0.06 ± 0.00
14	Isopentyl pentanoate	1097	0.07 ± 0.00
15	Isoamyl isovalerate	1103	0.85 ± 0.05
16	Methyl octanoate	1121	0.36 ± 0.03
17	Isobutyl hexanoate	1147	0.18 ± 0.02
18	Ethyl octanoate	1193	6.96 ± 0.60
19	ethyl E-2-octenoate	1244	0.18 ± 0.04
20	Isopentyl hexanoate	1247	0.66 ± 0.12
	Total esters		50.02 ± 1.00
	Terpenes		
21	Limonene	1028	5.04 ± 0.02
22	(E)-β-Ocimene	1034	3.31 ± 0.03
23	(Z)-β-Ocimene	1046	16.83 ± 0.25
24	γ-Terpinene	1057	0.11 ± 0.01
25	α-Terpinene	1126	0.15 ± 0.01
26	Mentha-1,4,8-triene	1129	0.54 ± 0.05
	Total terpenes		25.98 ± 0.32
	Acids		
27	Acetic acid	703	14.06 ± 0.10
28	Butanoic acid	792	1.66 ± 0.13
29	3-Methyl-Pentanoic acid	845	2.15 ± 0.03
30	2-Methyl butyric acid	851	0.17 ± 0.00
	Total acids		18.04 ± 1.10
	Alcohols		
31	2-Methyl-3-Hexanol	950	0.49 ± 0.03
32	2-Methylpentanol	1010	0.08 ± 0.00
	Total alcohols		0.57 ± 0.03
	Ketones		
33	3-hydroxy-2-butanone	739	1.84 ± 0.08
34	3-Penten-2-one	756	2.74 ± 0.09
	Total ketones		4.58 ± 0.18
	Pyrazines		
35	Piperazine	909	0.14 ± 0.00
36	2-Ethyl-3,5-dimethylpyrazine	1076	0.16 ± 0.01

	Total pyrazines		0.30 ± 0.01
	Others		
37	3-Methylbutanal	717	0.14 ± 0.00
38	Allyl ethyl ether	720	0.10 ± 0.00
39	Toluene	775	0.13 ± 0.02
40	Dodecamethylcyclohexasiloxane	1298	0.13 ± 0.05
	Total others		0.50 ± 0.08

* Data were expressed as means ± standard deviation (SD)

4. Conclusions

The results demonstrated that PPF has a potential to be used in food formulations as a natural ingredient to improve nutritional value, technological and functional properties. PPF presented high levels of dietary fiber, carotenoids, lipids, energy value, oleic acid, palmitic acid, good fatty acids composition and volatile profile. Furthermore, PPF showed high levels of K, Mg, phenolic compounds and high antioxidant activity.

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ARTICLE 2: Effect of pequi (*Caryocar brasiliense* Camb.) flour incorporation on the quality of chicken nuggets.

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(Elaborated in accordance to the Journal of Meat Science)

Abstract

The effect of pequi flour (PF) (0%, 3%, 6%, 9% and 12%) on the physicochemical properties, and sensory quality of chicken nuggets was investigated. In general, all the variables studied were affected by PF ($p<0.05$). Increasing PF increased the values of dietary fiber, lipids, emulsion stability, cooking yield, redness (a*) and yellowness (b*) color, while reducing the values of moisture, protein, lightness (L*) color, ash, pH, texture profile. PF had no significant effect on the oil absorption, mass gain in the coating (pick-up) and water activity values. During storage time the hardness, springiness, cohesiveness and chewiness increased, while the redness (a*) and yellowness (b*) decreased; no significant changes were observed for L* values. The TBARS levels increased over the storage time, however, the values were within the acceptable limit (< 1.0 mg MDA/kg) for good quality meat product. Incorporation of PF up to 9% was more preferred by consumers, thus suggesting that PF is a novel and appropriate food matrix to develop functional chicken nugget.

Keywords: Meat product, dietary source, sensory properties, functional food, CATA test.

1. Introduction

Meat and meat products are one of the most nutritious food that represent a significant dietary source in the global human diet (Younis et al., 2022), and their consumption will continuously increasing, especially in the developing countries with population growth (Llauger et al., 2021). By 2050, the average global demand for meat products will increase approximately from 1.4 to 2.0 billion tones if the per capita consumption remains the same, assuming no change in population (Thangavelu et al., 2022).

Meat and meat products are excellent sources of high-quality protein with high biological value, vitamin B12 and other B complex vitamins, vitamin A, minerals such iron, zinc, selenium, and phosphorus, essential amino acids and fatty acids (Bohrer, 2017; Grasso et al., 2022). Among meat products, poultry meat is widely consumed throughout the world, owing to its good nutritive value, high production efficiency, low cost availability and for being a cheap source of high-quality protein that is efficiently absorbed by the human bord (Nawab et al., 2018).

Among poultry meat products, chicken nugget is widely consumed for its unique sensory characteristics, but it is also known for being convenience food, cheaper for consumers and easy to prepare (Echeverria et al., 2022; Ghasemi et al., 2022). Despite being nutritious and having all the above positive effects, chicken nugget like all other meat products is deficient of essential dietary fibers (Das et al., 2020), and bioactive compounds such as carotenoids and phenolics compounds. Apart from that, the presence of high levels of saturated fat acids and cholesterol (Paglarini et al., 2018) has been associated with increased risk of non-communicable diseases including type 2 diabetes and cardiovascular disease (Domingo & Nadal, 2017).

Thus, considering the growing concern about the correlation between food and health, the reformulation of highly consumed foods, such as chicken nuggets, by the incorporation of bioactive active compounds, represent a potential alternative to improve the nutritional value and an opportunity to promote a healthier diet, without requiring the consumers to change their dietary habits (Carpentieri et al., 2022). Recently, inclusion of vegetables fiber source into meat products has increased due to their health promoting effect of reducing the risk of diseases like constipation, coronary heart diseases, irritable bowel syndrome, inflammatory bowel disease and colorectal cancer (Illippangama et al., 2022). Moreover, incorporation of dietary fibers into meat products improves the techno-functional properties such as water holding capacity, emulsion stability and cooking yield, texture, and rheological properties (Talukder, 2015).

Because of those mentioned benefits, several studies have been conducted to reformulate chicken nuggets with different fiber-rich ingredients, such as amaranth flour (Tamsen et al., 2018), dragon fruit peel (Madane et al., 2020), eggplant powder (Akesowan & Jariyawaranugoon, 2021), green peas (Zaini et al., 2021), rice bran (Chayawat & Rumpagaporn, 2020) green banana and soybean hulls flours (Kumar et al., 2013), okara and rice bran (Echeverria et al., 2022) and chia flour (Barros et al., 2018). However, to our knowledge, no much information is available about the utilization of pequi (*Caryocar brasiliense*) pulp flour in chicken nuggets.

Pequi (*Caryocar brasiliense*) fruit is a rich source of fibers, minerals such as zinc, iron and phosphorus, fatty acids, especially oleic acid, and bioactive compounds including phenolics, tocopherols, phytosterols and carotenoids (Pinto et al., 2018; Silva et al., 2022; Torres et al., 2016). The presence of bioactive compounds in pequi has been highly correlated with various biological properties such as antioxidant and inflammatory properties, anticarcinogenic effects and antibacterial activities (Brito et al., 2022; Pereira et al., 2020; Torres et al., 2016).

Previous study has shown that the addition of pequi flour in chicken nuggets can improve the nutritional and technological properties of these products without drastically affecting the sensory characteristics (Braga-Souto et al., 2020). However, the effect of pequi flour incorporation on the color, instrumental texture profile, lipid oxidation and sensory attributes have not previously been assessed. Therefore, this study aimed to investigate the effect of different levels (0%, 3%, 6%, 9% and 12%) of pequi flour incorporation on the proximate composition, color, pH, instrumental texture profile, lipid oxidation and sensory attributes.

2. Material and methods

2.1. Materials

Frozen pequi fruit (*Caryocar brasiliense*) without the exocarp (a greenish-brown outer skin) and the white external mesocarp, harvested in 2021, were obtained from two Fruit Pulp Industries-Barra do Garças (Mato Grosso State, Brazil) and Goiânia (Goias State, Brazil). The fruits were then transported to the Pilot Plant in the Laboratory of Post-Harvest of Fruit and Vegetables Fruit of Federal University of Lavras (UFLA, Lavras, Brazil), where the experiment

was carried out. All the ingredients, including the chicken breast fillet were obtained from local markets in Lavras, Brazil.

2.2. Preparation of pequi flour (PF)

Fruits consisted of the internal mesocarp (yellowish-orange pulp) and seeds, without any physical injury and uniform in size and color were visually selected, sanitized with sodium hypochlorite solution (100 mg/L) for 15 min and then rinsed in distilled water to remove the residual chlorine. Afterward, they were bleached in a water bath at 80 ^{o}C for 8 min. After 10 min cooling over in an ice bath, the fruits were then manually pulped using the stainless knife and the pulp and seeds were removed. Subsequently, the pulp was dried in an oven with forced air circulation (Solab SL 102 model, Piracicaba, São Paulo, Brazil) at 40^{o}C for 30 h. The dried pulp was first crushed using an industrial blender (Poli model LS-06, Brusque/Brazil), and then in a knife mill (Tecnal T650 model, Piracicaba, Brazil) and passed through a 20 – mesh sieve (0,85mm) to obtain the PF. The resulted PF was vacuum-packed in polyethylene plastic bags containing approximately 500g and stored frozen at -20 ^{o}C until further use.

2.3. Preparation of chicken nuggets

Five different formulations (Table 1) in three separated batches (repetitions) were prepared in the pilot plant of Laboratory of Meat Science and Technology (LabCarnes) at Federal University of Lavras. Briefly, frozen chicken breast fillets were thawed at 4 ^{o}C overnight, cut into smaller chunks and ground twice in an electric meat grinder (BECCARO model PB-22, Rio Claro, Brazil), first with an 8 mm disc and then with 6 mm disc and homogenized with all the other ingredients (Table 1). The final mixture was molded in a hamburger mold (11 cm diameter), immediately frozen at -18 ^{o}C for 8h, cut into four equal pieces (2.5 x 2.5 x 1.0 cm) and refrozen for 1h. After this, the formulated nuggets were predusted with wheat flour, batter-coated with a mixture comprised of wheat flour (16.5%), corn starch (10.0%), powdered milk (6.5%), salt (1%) and ice water (66.0%), and breaded with dried bread crumbs. After coating, the chicken nuggets were pre-fried in soybean oil at 90 ^{o}C for 30 s, immediately removed from the fryer and drained on absorbent papers at room temperature for 10min to remove the excess surface oil. Finally, the chicken nuggets samples were stored in polyethylene plastic bags and frozen at -18 ^{o}C for 45 days. The physicochemical analyses were carried out at 0, 15, 30 and 45 days of frozen time and the sensory evaluations were performed only at first day of storage period.

Table 1 Formulations of the chicken nuggets

Ingredients (%)	Treatments				
	PF0%	PF3%	PF6%	PF9%	PF12%
Chicken breast fillet	81.0	78.0	75.0	72.0	69.0
Pequi flour	0.0	3.0	6.0	9.0	12.0
Water	15.0	15.0	15.0	15.0	15.0
Salt (NaCl)	1.0	1.0	1.0	1.0	1.0
Phosphate	0.5	0.5	0.5	0.5	0.5
Textured soy protein	1.4	1.4	1.4	1.4	1.4
Sodium erythorbate	0.3	0.3	0.3	0.3	0.3
Garlic powder	0.3	0.3	0.3	0.3	0.3
Onion powder	0.3	0.3	0.3	0.3	0.3
White pepper powder	0.1	0.1	0.1	0.1	0.1
Nutmeg powder	0.1	0.1	0.1	0.1	0.1

PF0%, chicken nuggets without pequi flour (control); PF3%, PF6%, PF9% and PF12%, chicken nuggets with 3, 6, 9 and 12% pequi flour, respectively.

2.4. Heat treatment of chicken nuggets

Frozen pre-fried chicken nuggets samples were thawed (1h) at room temperature and deep-fried using 2.5 L soybean oil in a pre-heated domestic deep fryer at 180 ^{o}C for 4 min (the samples were turned over every minute). The heat temperature and cooking time were chosen based on the preliminary experiments. After cooking, all samples were cooled down to the room temperature and used for the analyses.

2.5. Experimental design

The effects of PF (0%, 3%, 6%, 9%, 12%) on the proximate composition variables, energy value, oil absorption, pick-up, water activity, and pH value were assessed using a completely randomized design (CRD), in three replicates. Data from instrumental color, texture profile analysis and TBARS were arranged in 5 x 4 factorial design, with five levels of PF and four levels of storage time (0, 15, 30, 45 days). Data from sensory analysis were evaluated using a randomized block design (RBD), including assessors as a random effect (repetitions).

2.6. Analyses

2.6.1 Proximate composition

The proximate composition analysis was carried out in triplicate on raw chicken nuggets. The moisture, protein, ash and total dietary fiber were performed according to previous published methodologies (AOAC, 2019). For total moisture content (AOAC 950.46B), 10 g of the sample were dried to constant weight at 105 ^{o}C. Protein content (AOAC 981.10) was determined by the Kjeldahl method and the factor 6.25 was applied for conversion of nitrogen to crude protein. The ashes were analyzed by combustion at a temperature of 550 ^{o}C (AOAC 920.153). The fat content was analyzed by the cold extraction (chloroform/methanol/water) according to Bligh & Dyer (1959). The total dietary fiber content was analyzed by the enzymatic-gravimetric method (AOAC 991.43) using a Total Dietary Fiber Assay Kit. Non-fiber carbohydrate content was determined by subtracting the sum of moisture, protein, fat, ash, and total dietary fiber from 100%. Energy was calculated using the Atwater conversion factors (4 for carbohydrate and protein and 9 kcal for fat). The outcomes were express in fresh matter.

2.6.2. Percentage of mass gain in the coating (pick-up) and oil absorption

The pick-up was obtained by the difference between the mass of nuggets (10 samples per treatment) before and after the three-step coating process (pre-dust, batter, and breading) following the equation (1). Oil absorption was estimated by the difference between the mass of the pre-fried and raw samples according to equation (2).

$$\text{Pick-up (\%)} = \left(\frac{\text{Nuggets with coating - Nuggets without coating}}{\text{Nuggets with coating}} \right) \text{x } 100 \qquad (1)$$

$$\text{Oil absorption (\%)} = \left(\frac{\text{Weight pre-fried nuggets - Weight raw nuggets}}{\text{Weight pre-fried nuggets}} \right) \text{x } 100 \qquad (2)$$

2.6.3. pH and water activity

The pH and water activity (a_w) were carried out in triplicates, in raw samples, at zero storage period. 5g of nugget samples were homogenized with 45 mL of distilled water for 2 min and the pH was determined using a pH meter (Tecnal TEC-3MP). Water activity of the nuggets was measuring using an Aqualab Water Activity Meter (Decagon Devices, Inc., Pullman, WA, USA) at 25 ^{o}C.

2.6.4. Texture profile analysis and instrumental color

The instrumental texture profile analysis (TPA) parameters (hardness, springiness, cohesiveness and chewiness) were investigated using a Texture Analyzer (TA-XT2i Stable Micro Systems, Godalming, UK) equipped with a 6 mm diameter cylindrical aluminum probe, at a constant speed of 2 mm/s and the samples were compressed twice to 50% of their original height with a compression load cell of 5kg. Prior to each analysis, the samples were cooled down to room temperature for 30 min, cut into cubes of 2 cm edge and the coating system was removed to avoid the potential interference on the results of texture parameters and to evaluate the effect of the PF on the internal color of the chicken nuggets.

The effect of the PF on the internal color of the formulations were assessed using a Minolta CR-400 (Konica Minolta Sensing, Inc., Osaka, Japan) colorimeter, operating with D65 illuminant, 8 mm aperture, and 10^{o} observer angle. The color indexes of the CIElab system L* or lightness (L* 0 = black; 100 = white), a* (redness) and b* (yellowness) were recorded at 10 points for each treatment.

2.6.5. Lipid oxidation

Lipid oxidation of samples during storage period was performed using the thiobarbituric acid reactive substances (TBARS) as described by Jo & Ahn (1998). TBARS values were determined using the standard curve generated by using 1,1,3,3, tetra ethoxypropane standard calibration curve and reported as mg malonaldehyde (MDA)/kg sample.

2.6.6. Sensory analysis

Prior to sensory analysis, the research was approved by the Human Research Ethics Committee of the Federal University of Lavras (CAAE: 55582822.6.0000.5148) and all panelists signed a free and informed consent form, agreeing voluntarily to participate in the study. The volunteers among undergraduate and post-graduate students, staff and professors of the Federal University of Lavras were selected on the basis of their interest, availability and habit in consuming chicken nuggets, at least once a month. The sensory evaluation was performed through the descriptive test check-all-that apply (CATA), acceptance and purchase intention tests as described by Guimarães et al. (2022), with minor changes. For selecting the CATA descriptors, a preliminary test with a focus group of 19 panelists of both genders, was performed. Each consumer individually tasted the samples, followed by discussion in a group

until reach a consensus about the descriptors. The final descriptors terms were grouped into 5 categories including appearance (12), aroma (7), flavor (4), taste (3), and texture (6) (Table 2).

A total of 75 untrained panelists were randomly recruited at UFLA to perform the sensory analysis. Among them, 64% declared themselves female, and 36% male. The voluntaries included 61.3% post-graduate students and 38.7% undergraduate students with ages ranging from 18 to 60 years. More than 80% of the panelists had a family income in range from 1 to 4 minimum wages. A total of 52% stated that used to consume chicken nuggets once a month, 32% twice a month, 4% twice a week and 12% once a week. The tests were carried out in individual booths under fluorescent lighting. The samples were deep-fried and kept in a styrofoam tray until served to panelists. The samples were cut into two pieces, coded with random three digits numbers and assessed in randomly in a monadic order. The assessors were required to drink water between each sample evaluation for palate cleansing. Before the analysis, the consumers were instructed to read the descriptors terms, and after they tried the samples, they were asked to select without limit all the descriptors they considered appropriate to describe each sample. For the acceptance test, the attributes appearance, aroma, taste, texture, and overall acceptance were assessed using a structured 9-point hedonic scale (1 = "dislike very much" to 9 = "like very much"). Besides, a 5-point hedonic scale (1 = certainly would not buy; 5 = certainly would buy) was also used to evaluate the purchase intention of the chicken nuggets.

2.7. Statistical analysis

Data were reported as mean ± standard deviation. Analysis of variance (ANOVA) was carried out using R version 4.0.4 (R Core Team, 2020). Significant differences between means were identified using Tukey's test. Correspondence Analysis (CA) was used to evaluate the descriptors terms of CATA questions, using SPSS version 24.0 (SPSS Inc., Chicago, IL, USA). Cochran's Q test was applied to identify significant differences between treatments for each descriptor term. For all analyses, the significance level was fixed at $p < 0.05$.

3. Results and discussion

3.1. Physicochemical and techno-functional properties

Table 2 presents the proximate composition, energy value, emulsion stability, oil absorption, pick-up, aw and pH. Except for oil absorption and pick-up, all other variables were

significantly affected by the addition of pequi flour in chicken nuggets different ($p < 0.05$). In general, increasing concentrations of pequi flour resulted in a decrease in moisture content from 65.78 to 62.85 g/100g. However, there was no significant difference in moisture content between the formulations with 3 and 6% of pequi flour ($p > 0.05$). The decrease in moisture content was due to the increasing level of pequi flour associated with its low moisture content (6.72g/100g). The decrease trend in moisture content was also reported by Barros et al. (2018) who replaced chicken skin (0-20%) with chia flour aiming to produce a fibre-enriched chicken nugget with a healthier fatty acid profile.

The fat content and energy value of nuggets increased with the increasing concentrations of pequi flour, as was expected, due to the high fat content of pequi flour (66.40 g/100g) (Silva et al., 2022). Regarding protein, the addition of pequi flour up to 12% to chicken nugget decreased the protein content compared to the control sample (ranging from 13.95 to 7.45 g/100g), which could be due to lower protein in pequi flour than chicken meat. This behavior was also observed by Zaini et al. (2021) who reported a reduction of protein content in chicken nugget as the level of green peas powder increased (0-12%).

A reduction in ash content of the treated nuggets was observed. As the level of pequi flour increased, the ash content decreased from values ranged between 2.20 to 1.86g/100 compared control samples (2.42g/100g). This reduction in ash content could be associated with the incorporation of pequi flour in chicken nuggets. Analogous observations have been found by Verma et al. (2015) and Zaini et al. (2021) in low-fat chicken nuggets formulated with pea hull flour and chicken nuggets with added green peas powder, respectively.

As the incorporation level of pequi flour increased from 3% to 12%, the Total Dietary Fiber (TDF) of the chicken nuggets also increased significantly ($p < 0.05$), whereas the content of non-fiber carbohydrate changed slightly (Table 2). In all the samples formulated with pequi flour the increment in dietary fiber ranged from 0.95 to 1.71 g/100g, while in control samples this content was significantly lower (0.65 g/100g). On the other hand, the non-fiber carbohydrate content ranged between 3.26 and 3.72 g/100g. The increase of the dietary fiber with the addition of pequi flour was due to its high dietary fiber content (12.56 g/100g) (Silva et al., 2022). The results of this study are in accordance with those observed by Echeverria et al. (2022), who noted that addition of two byproducts, okara flour and rice bran into chicken nuggets, as substitutes for chicken skin increased the contents of dietary fibers by nearly 100%.

In recent years, consumption of meat products fortified with dietary fiber has been linked to potential health benefits, contributing to lowering cholesterol and preventing the risk of coronary heart diseases, constipation, diabetes, obesity, and some types of cancer (Aslam et al., 2014; He et al., 2022). Moreover, dietary fibers besides offering health benefits, their functional and technological properties help to improve various quality parameters of meat products including cooking yield, juiciness, shrinkage, water-holding capacity, oil absorption, and emulsion stability (Younis et al., 2022). This finding suggests that pequi flour can be considered a promising natural functional ingredient source of dietary fiber in chicken nugget formulation.

Incorporation of pequi flour had no significant effect ($p > 0.05$) on oil absorption (Table 2). Conversely, Tamsen et al. (2018) observed an increase in oil absorption as the proportion of amaranth in the formulation of chicken nuggets increase.

Referring to the pick-up values, no significant differences ($p > 0.05$) were observed among the control samples and reformulated samples, which was expected since the formulation changes were made out only in the meat batter and not in the coating system. The pick-up values ranged between 29.73 and 31.70%, which were very close to the 30%, suggested by the coating system manufacturer. Similar results were reported in the study conducted by Barros et al. (2018) in which different levels of chia flour were incorporated into chicken nuggets.

Regarding water activity (a_w), no significant differences ($p > 0.05$) were observed by the increasing proportion of PF up to 6%, but the mean aw values reduced in nuggets formulated with 9% PF or more ($p < 0.05$). Similarly, Barros et al. (2018) observed a decrease in a_w values as the percentages of pequi flour in the formulation of chicken nuggets increased.

In the case of pH, it could be seen that with the increase of PF in chicken nuggets, the pH values significantly decreased (ranging from 6.28 to 6.03), which was in line with previous finding by Pires et al. (2020) who also observed a reduction in pH values in bologna type sausages with added chia flour. Choi et al. (2011) stated that incorporation of fiber can enhance or reduce the pH values of meat products, depending on the type of fiber. Thus, it is reasonable to deduce that the pH reduction observed in the present study, might be related to the lower pH of the pequi flour added (4.25) in comparison with the pH of meat.

Table 2 Effects of different levels of pequi flour (PF) on the proximate composition, emulsion stability, oil absorption, pick-up, a_w and pH of chicken nuggets.

Analysis	Treatments					Sig
	PF0%	PF3%	PF6%	PF9%	PF12%	
Proximate composition (g/100g)						
Moisture	65.78 ± 0.24^{b}	66.30 ± 0.15^{a}	66.41 ± 0.07^{a}	64.96 ± 0.13^{c}	62.85 ± 0.19^{d}	*
Lipids	13.95 ± 0.98^{d}	15.03 ± 0.52^{d}	17.10 ± 0.37^{c}	19.46 ± 0.59^{b}	22.41 ± 0.91^{a}	*
Protein	13.93 ± 1.00^{a}	13.23 ± 0.50^{a}	10.67 ± 0.55^{b}	8.84 ± 0.09^{bc}	7.45 ± 0.92^{c}	*
Ash	2.42 ± 0.09^{a}	2.20 ± 0.12^{ab}	2.10 ± 0.04^{bc}	1.92 ± 0.07^{cd}	1.86 ± 0.09^{d}	*
Total dietary fiber (TDF)	0.65 ± 0.03^{e}	0.95 ± 0.02^{d}	1.16 ± 0.02^{c}	1.33 ± 0.04^{b}	1.71 ± 0.09^{a}	*
Carbohydrate	3.26 ± 0.18^{abc}	2.30 ± 0.16^{c}	2.55 ± 0.14^{bc}	3.50 ± 0.67^{ab}	3.72 ± 0.45^{a}	*
Energy value (Kcal/100g)	194.35 ± 4.89^{d}	197.39 ± 2.34^{d}	206.81 ± 1.66^{c}	224.47 ± 2.30^{b}	246.38 ± 3.84^{a}	*
Oil absorption (%)	3.02 ± 0.61	2.77 ± 0.22	2.99 ± 0.37	2.67 ± 0.44	3.06 ± 0.28	n.s.
Pick-up (%)	29.73 ± 1.07	30.24 ± 2.20	29.37 ± 2.91	31.06 ± 2.77	31.78 ± 4.20	n.s.
aw	0.97 ± 0.01^{a}	0.98 ± 0.01^{a}	0.97 ± 0.00^{a}	0.96 ± 0.01^{b}	0.95 ± 0.01^{b}	*
pH	6.28 ± 0.02^{a}	6.21 ± 0.01^{b}	6.14 ± 0.01^{c}	6.13 ± 0.01^{c}	6.03 ± 0.01^{d}	*

Data are reported as means ± standard deviation (n = 3). PF: pequi flour; PF0%: control chicken nuggets with a traditional formula; PF3%, PF6%, PF9% and PF12%: samples with 3, 6, 9 and 12% pequi flour incorporation, respectively. Mean with different letters in a row are significantly different (*$p < 0.05$) by Tukey test. n.s – non-significant.

3.2. Instrumental color and textural properties.

Color is one of the most important attribute of meat product, which has remarkable effect on the consumer's perception of quality (Altmann et al., 2022). As shown in Fig. 1, color variables lightness (L*), redness (a*) and yellowness (b*) were significantly affected by both the concentrations of PF as well as the storage time ($p < 0.05$). L* values significantly declined while a* and b* values were significantly increased by increasing the amount of PF in chicken nuggets compared to control samples, regardless the storage time (Fig. 1A). In all treatments, the lowest L* values and the highest a* and b* values were recorded at higher concentrations of pequi flour (12%). These variations have been mainly attributed to orange-yellow color of pequi flour, which gave the nuggets an orange-yellow appearance. Silva et al. (2022) have found that pequi flour had considerable levels of carotenoids which are responsible for yellow-orange color. Calvo et al. (2008) and Qiu & Chin (2021) also observed a decrease in L* values and increase of a* and b* values in raw and cooked pork patties with added grape tomato powder and dry fermented sausages enriched with lycopene from tomato peel, respectively. During the whole storage time, in general, it was observed that the L* values of all treatments did not differ significantly from each other, although a slight decrease between 30 and 45 days was detected for nuggets formulated with 0 and 3% of PF. This decrease trend might be related to water loss during frozen storage, and subsequent decreases in surface light reflectance and L* value (Hughes et al., 2014). On the other hand, the a* and b* values of all treatments (Fig. 1B and C), in general, were found to decrease throughout the storage period, with the exception of nuggets without PF (0% PF) which decreased up to 30 days followed by increase thereafter. This results might be related to the oxidation of lipids and meat pigments that occur simultaneously and one increases the other (Bellucci et al., 2022). Additionally, the cooking process might also have impacted to the both protein and lipid oxidation

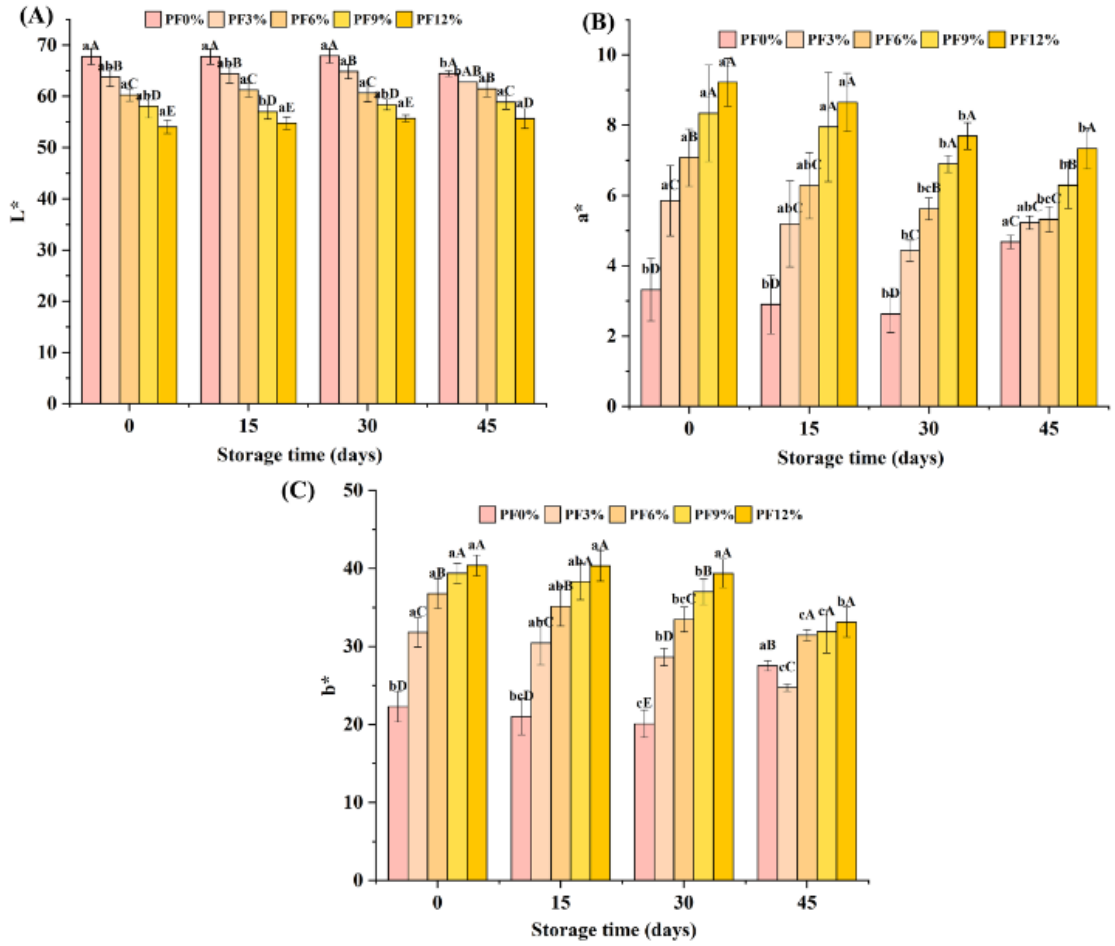


Fig.1. Effects of different levels of pequi flour on objective color (L*, a* and b*) of the chicken nuggets during storage for 45 days. PF: pequi flour; PF0%: control chicken nuggets with a traditional formula; PF3%, PF6%, PF9% and PF12%: samples with 3, 6, 9 and 12% pequi flour incorporation, respectively. Different letters (A-D) indicate significant differences between different levels of pequi flour (bars with different colors) for the same storage time ($p < 0.05$), while different letters (a-c) indicate significant differences between different storage times for the same level of pequi flour (bars with the same color) ($p < 0.05$) by Tukey test.

Texture profile analysis (TPA) is a crucial tool for food quality used to investigate the parameters such as hardness, springiness, cohesiveness, and chewiness (Zangirolami et al., 2023). Hardness (N) represents the peak force required to compress the sample; springiness (mm) is the ability of sample to reconstruct its initial form after a deforming force was eliminated; cohesiveness shows how strong the sample resists a second deformation relative to the initial one; chewiness stimulates the chewing and represent the work necessary to masticate the sample form swallowing. In this study, it was observed that incorporation of PF and storage had a significant ($p < 0.05$) effect on TPA properties (Fig. 2). In general, all the TPA variables decreased with the increasing levels of PF and during storage time, with the lowest values observed at higher concentrations (12% of PF). This suggests that the incorporation of PF in

the chicken nuggets improved the fluidity and softened them. These outcomes agreed with a previous study by Madane et al. (2020), who reported a reduction of hardness, cohesiveness, gumminess and chewiness values with the increment of dragon fruit peel powder in chicken nuggets. Furthermore, Das et al. (2015) found that hardness, chewiness, and gumminess values of goat meat nuggets decreased with the addition of bael pulp residue. Our findings, however, contradict with the results found by several authors (Echeverria et al., 2022; Tamsen et al., 2018; Zaini et al., 2021) who reported an increase of all TPA variables with increasing of plant-based materials in chicken nuggets. These differences could be attributed to various factors, including processing and storage conditions, plant-based characteristics and chemical composition, as well as the emulsion properties and the other ingredients used in the formulation of nuggets. In the present study, the reduction of TPA variables might be related to the higher fat content from PF (66.40 g/100g), which can act as softness agent in the chicken nugget protein matrix, resulting in the instability of protein structure. Additionally, the low pH of PF might have induced high protein denaturation leading to reduce the TPA variables by the damaged of the continuous protein gel network.

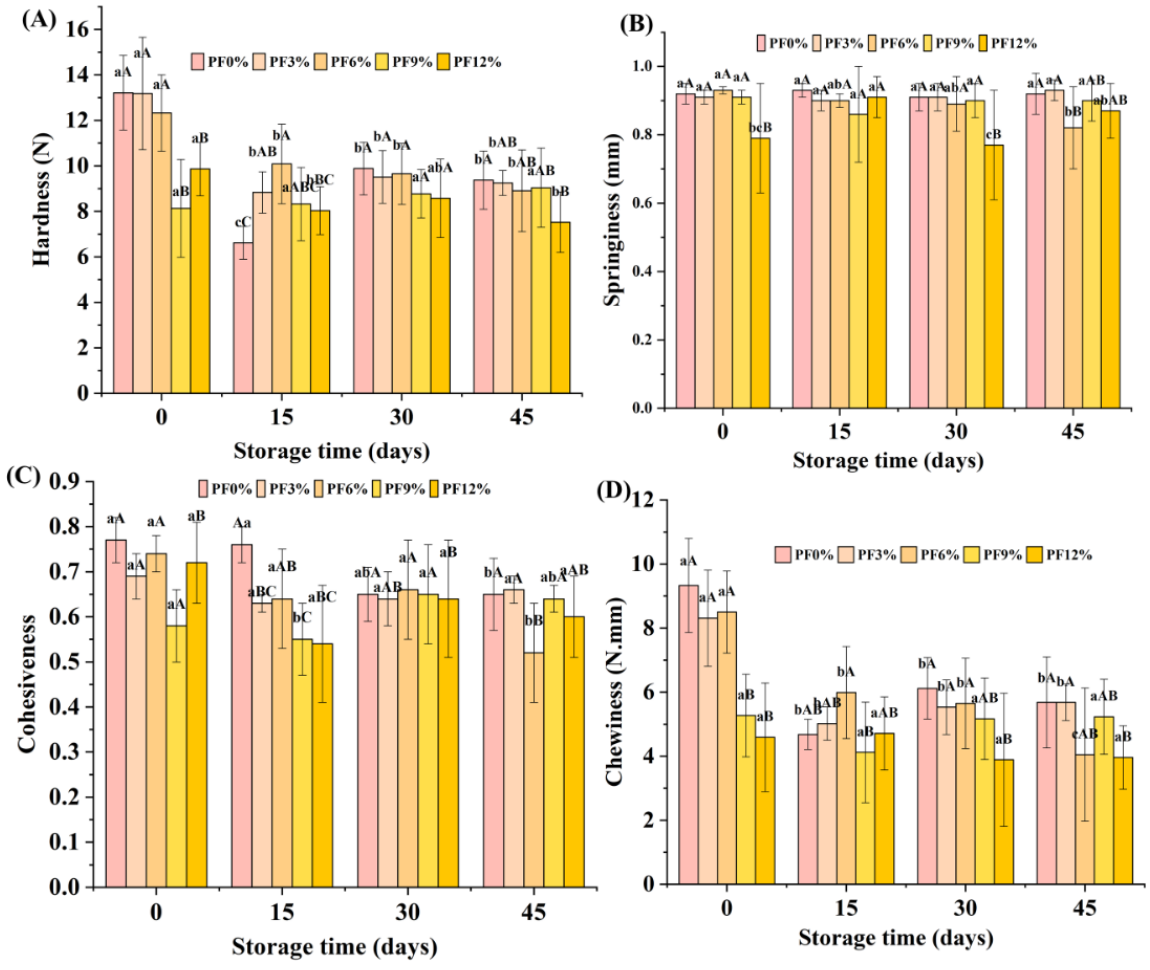


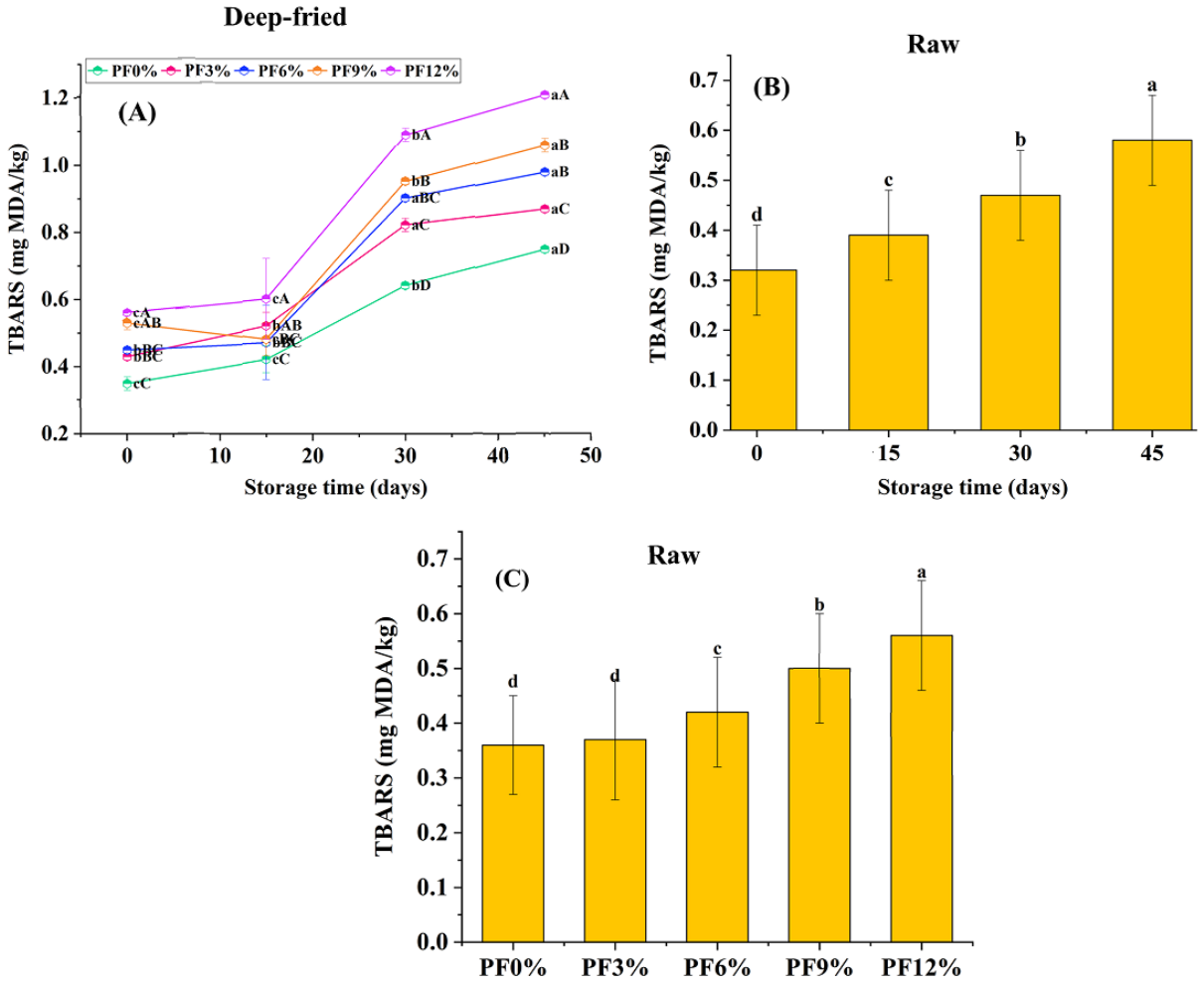
Fig.2. Effects of different levels of pequi flour and storage time for 45 days on the texture profile parameters of the chicken nuggets. PF: pequi flour; PF0%: control chicken nuggets with a traditional formula; PF3%, PF6%, PF9% and PF12%: samples with 3, 6, 9 and 12% pequi flour incorporation, respectively. Different letters (A-C) indicate significant differences between different levels of pequi flour (bars with different colors) for the same storage period ($p < 0.05$), while different letters (a-c) indicate significant differences between different storage times for the same level of pequi flour (bars with the same color) ($p < 0.05$) by Tukey test.

3.3. TBARS values

Lipid oxidation is one of the most important factors affecting the shelf life and quality of meat product. This process leads to the development of off-flavors and oxidative rancidity in meat products. The rancidity level in meat and meat products can be assessed by the TBARS values (Qiu & Chin, 2022). As shown in Fig. 3A, B and C, the TBARS values in raw and cooked chicken nuggets were significantly affected by the incorporation of PF and storage time ($p < 0.05$). There was an interaction between treatments and storage time for cooked samples, whereas no interaction was found between these two factors in raw chicken nuggets. TBARS contents of chicken nuggets with PF were significantly higher than of the control samples. Increasing the levels of PF significantly increased the TBARS values of all samples during the storage period, and as expected, TBARS values of cooked chicken nuggets were higher than those of raw chicken nuggets. These results indicated that incorporation of PF in chicken nuggets was not effective to retard lipid oxidation and lower TBARS values. The outcomes are in agreement with those of Xiong et al. (2022) who found that addition of sorghum bran in beef sausages significantly increased the TBARS values and promoted lipid oxidation. Mantihal et al. (2021) also reported an increase of TBARS contents in chicken patties incorporated with round cabbage powder during storage. Meat lipid oxidation is a complex process affected by several factors, including temperature, cooking and storage conditions, and meat composition such as lipid content and fatty acid composition (Domínguez et al., 2019). Pequi, besides being a good source of bioactive compounds such as phenolics, tocopherols, phytosterols and carotenoids (Cornelio-Santiago et al., 2022), also contains high levels of unsaturated fatty acids, mainly oleic acid (Pinto et al., 2018), which are highly susceptible to oxidation and may have contributed to increase the TBARS values, resulting in lipid oxidation of chicken nuggets. In this view, we can speculate that the antioxidant action of phenolic compounds was probably neutralized by the pro-oxidant effect promoted by PF unsaturated fatty acids.

Until 45th day of storage, the TBARS values of the raw and cooked chicken nuggets were in range from 0.32 to 0.58 mg MAD/kg and from 0.35 to 1.21 mg MAD/kg, respectively

(Fig. 3A, B and C). However, it is important to highlight that these values were below the acceptable minimum limit of 2.5 mg of MAD/kg of the samples established by Qiu & Chin, (2021) which is the detectable concentration for rancidity.



3.4. Sensory analysis

As shown in Fig. 4B and C, incorporation of PF resulted in significant differences ($p < 0.05$) on the sensory properties of chicken nuggets. Regardless the percentage level of PF, all the samples were preferred in terms of appearance, aroma, and texture (Fig. 4B), with mean sensory scores (7.08 to 7.93) located between the 9-point hedonic scale: “I liked moderately” and “I liked very much”, being the control (PF0%) samples with the highest notes followed by the treatments PF9%, PF6% and PF3%, while the treatment PF12% exhibited the lowest scores. Regarding the taste, there were no significant differences ($p > 0.05$) between the control samples (PF0%), treatments PF3% and PF9%, and all these treatments were more ($p < 0.05$) acceptable than PF6% and PF12%, with scores ranging from 7.53 to 7.93 (from I liked moderately to I liked very much), whereas the treatment PF12% had the lowest taste score of 5.76. Concerning

overall acceptance (Fig. 4B), the control samples and the treatments with up to 9% pequi flour were well accepted with scores between 7.09 to 7.69 (from I liked moderately to I liked very much), and the treatments with 12% of PF had the lowest overall acceptance scores. The results of purchase intention (Fig. 4C) showed similar behavior with the overall acceptance and in all samples the mean scores were in the range from 3.19 to 4.33, being the control samples and the treatment PF9% with the highest scores between "would probably buy (4)" and "would certainly buy (5)". However, the treatments PF3%, PF6% and PF9% were not significantly different PF9%, and PF3%, PF6%, while the chicken nuggets treated with 12% of pequi flour had the lowest purchase intention scores. The lowest acceptance for the treatment PF12% might be related to the perception of yellow color, and intense pequi flavor and aroma associated with high levels of PF which, as noted in the CATA analysis. These outcomes suggest that PF up to about 9% can be incorporated in the chicken nuggets without compromising their acceptability by consumer in comparison with the control samples.

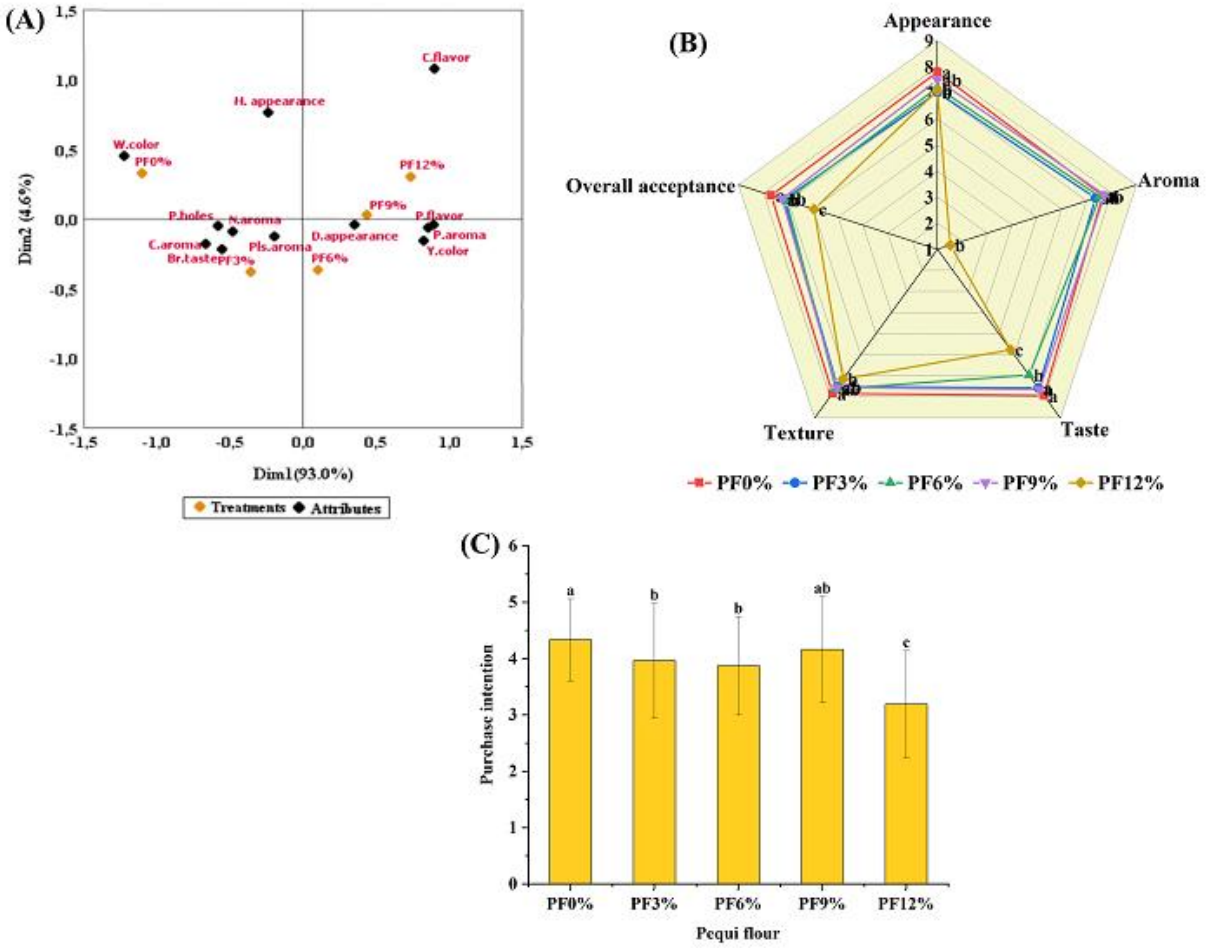


Fig.4. Correspondence analysis map of the CATA descriptors terms in the first two dimensions (A), and the effect of different levels of pequi flour (B and C) on the sensory attributes of chicken nuggets. PF: pequi flour; PF0%: control chicken nuggets with a traditional formula;

PF3%, PF6%, PF9% and PF12%: samples with 3, 6, 9 and 12% pequi flour incorporation, respectively. H. appearance: Homogeneous internal appearance; P.holes: presence of holes; W. color: white color external appearance; Y.color: yellowish color internal appearance; D.appearance: dry appearance; P.aroma: pequi aroma; Pls.aroma: pleasant aroma; C.aroma: chicken aroma; N.aroma: nugget aroma; P. aroma: pequi aroma; P.flavor: pequi flavor; C.flavor: chicken flavor; Br.taste: bitter residual taste. Different letters (a-c) indicate significant differences

The results (Table 6) of sensory analysis by the CATA test, showed that no significant differences ($p > 0.05$) were observed among the samples for most of the studied attributes, according to Cochran's Q test, indicating that these attributes did not play a crucial role for explaining the differences among the samples. The consumers were able to perceive ($p < 0.05$) differences only for 12 sensory descriptors including 5 appearance terms (homogeneous internal appearance, presence of holes, white color external appearance, yellowish color internal appearance and dry appearance), 4 aroma terms (pequi aroma, pleasant aroma, chicken aroma and nugget aroma), 2 flavor terms (pequi flavor and chicken flavor) and 1 taste term (bitter residual taste). In the present study, no differences were found ($p > 0.05$) for texture descriptors.

Table 3 Frequency citations of the check-all-that-apply (CATA) descriptors terms of deep-fried chicken nuggets with different levels of pequi flour.

Appearance terms	PF0%	PF3%	PF6%	PF9%	PF12%
Homogeneous internal appearance (H. appearance)***	44	19	22	30	34
Presence of holes (P.holes)**	35	25	21	16	15
Golden color external appearance^{ns}	40	43	51	48	50
Frying appearance^{ns}	32	38	44	42	50
White color external appearance (W. color)***	22	11	6	4	3
Nugget appearance^{ns}	47	37	39	33	33
Yellowish color internal appearance (Y.color)***	2	22	42	49	62
Dry appearance (D.appearance)*	11	16	19	24	27
Heterogeneous internal appearance^{ns}	8	15	17	17	15
Fibrous appearance^{ns}	9	8	4	5	6
Moist internal appearance^{ns}	25	20	24	16	18
Crunchy appearance^{ns}	40	35	44	45	47
Aroma terms					
Pequi aroma (P.aroma)***	0	20	33	43	58
Frying aroma^{ns}	42	49	46	39	46
Rancid aroma^{ns}	1	2	1	3	4
Spicy aroma^{ns}	2	1	3	5	7
Pleasant aroma (Pls.aroma) *	48	39	40	52	31
Chicken aroma (C.aroma)***	51	37	31	22	17
Nugget aroma (N.aroma)***	54	42	34	32	26
Flavor terms					
Pequi flavor***	1	26	38	59	67
Chicken flavor (C.flavor)***	62	51	40	31	27

Spicy flavor^{ns}	42	39	45	45	52
Pleasant flavor^{ns}	54	53	50	49	41
Taste terms					
Bitter taste^{ns}	1	2	3	3	7
Salty taste^{ns}	8	6	3	6	9
Bitter residual taste**	2	3	3	10	15
Texture terms					
Crunchy crust texture^{ns}	58	54	60	53	57
Soft texture^{ns}	47	47	59	46	44
Crumbly texture^{ns}	7	8	4	8	9
Fibrous texture^{ns}	15	15	8	9	21
Juicy^{ns}	32	20	18	23	22
Crunchy internal texture^{ns}	3	8	5	9	13

Data are reported as means ± standard deviation (n = 3). PF: pequi flour; PF0%: control chicken nuggets with a traditional formula; PF3%, PF6%, PF9% and PF12%: samples with 3, 6, 9 and 12% pequi flour incorporation, respectively. Significant difference for *$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$ (Cochran´s Q test)
^{ns} non-significant difference according ($p > 0.05$) according to Cochran's Q test.

In order to distinguish the chicken nuggets formulations, the correspondence (CA) analysis was performed with the 12 significant sensory terms generated by the CATA questionnaire and the results are presented in Fig. 4A. The first two dimensions explained 97.6% of the total variance of the experimental data (93% and 4.6%, respectively). According to the first dimension, the treatments were divided into two distinct groups. The first group (PF6%, PF9% and PF12%) was situated in the right quadrant and was characterized by dry appearance, yellowish color internal appearance, pequi flavor, pequi aroma and chicken flavor. The second group was formed by the PF0% and PF3% treatments, which were situated in the left quadrant and were associated with the terms: pleasant aroma, chicken aroma, presence of holes, nugget aroma, bitter residual taste, white color external appearance and homogeneous internal appearance. It should be noted that an increasing addition of PF resulted in a corresponding higher citation frequency of the terms “yellowish color internal appearance”, “pequi aroma and pequi” and “pequi flavor”. This is also in line with CA analysis where chicken nuggets with high levels of pequi flour (PF6%, PF9% and PF12%) were much closely associated with these attributes. These findings indicated that consumers were able to perceive the differences between the control samples and the treatments with PF.

4. Conclusions

This study demonstrated that PF has a potential to be incorporated in chicken nuggets as functional ingredient. Inclusion of PF significantly increased the values of dietary fiber,

lipids, and decreased the texture profile variables, including hardness, cohesiveness, springiness, and chewiness. The TBARS values increased with the incorporation of PF and during storage time, which is most likely due to the presence of high levels of oleic acid, which is susceptible to oxidation. Incorporation up to 9% of PF can be recommended without affecting the acceptability of the product.

Acknowledgments

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ARTICLE 3: Effect of pequi (*Caryocar brasiliense* Camb.) flour and different cooking methods on the carotenoids content, fatty acids profile, volatile compounds and sensory attributes of chicken nuggets.

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(Elaborated in accordance to the Journal of Meat Science)

Abstract

This study investigated the effect of three different concentrations of pequi flour (PF) (0%, 6% and 12%) and four cooking method (air-frying, deep-frying, oven-roasting and microwave) on the cooking yield, carotenoids, fatty acids profile, volatile compounds and sensory attributes of chicken nuggets. Overall, all the variables were affected by PF and cooking method ($p < 0.05$). Increasing PF resulted in significant higher cooking yield, carotenoids, saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), and lower polyunsaturated fatty acids (PUFAs). The most predominant volatile compounds (VCs) in raw chicken nuggets were terpenes and esters. Addition of PF significantly increased terpenes and esters contents, and decreased the contents of aldehydes, ketones and aliphatic hydrocarbons. Air-frying and oven-roasting exhibited the highest cooking yield. In addition, air-frying showed the highest contents of total carotenoids. Deep-frying revealed the highest PUFA and PUFA/SFA ratio values and the lowest SFA. Microwave and air-frying resulted in the highest MUFA contents. The health indicators for all cooking methods were within the acceptable limit. Incorporation up to 9% of PF did not interfere on the acceptability of the chicken nuggets. Deep-frying resulted in better sensory characteristics, whereas microwave was considered as the worst cooking method regarding the sensory attributes.

Keywords: Meat product, carotenoids, sensory properties, functional food.

1. Introduction

Over the last years, there has been a growing consumer demand for functional foods, due to increasing concern about the relationship between food and health among different socio-economic stratus around the world (Konar et al., 2022; Rios-Mera et al., 2021). This scenario has been magnified during the coronavirus disease 2019 (COVID-19) pandemic and is expected to remain high also within the post-pandemic era (Kaderides et al., 2021).

Since ancient times, meat and meat products have played a positive role in human nutrition because of their high nutritional value since they are good source of high-quality protein with a good balance of amino acids, fatty acids, B complex vitamins, minerals such as iron, zinc, phosphorus and selenium (Anzani et al., 2020; Karwowska et al., 2021). Nevertheless, the main drawback of meat and meat products is the deficiency of dietary fiber (Das et al., 2020) and the presence of saturated fat acids (Paglarini et al., 2020).

Among meat products, poultry has an important contribution on global consumption due to its low price and low fat content and for being an accessible source of protein (de Farias Marques et al., 2022; Evrendilek, 2022). One of the most produced and widely consumed chicken meat products is nugget. Chicken nuggets are highly appreciated for their characteristic texture and flavor, but they are also known as convenient, cheap, quick and easy to prepare foods (Echeverria et al., 2022; Ghasemi et al., 2022).

Considering the facts that meat is deficient in dietary fiber and chicken nuggets are one of the most widely consumed meat products owing to their low cost, practicality and convenience, attempts should be made to fortify these products in order to improve their nutritional value and render functional properties. In this regard, incorporation of functional ingredients in meat products such as chicken nuggets is one of the most useful strategies to promote healthier diet, without requiring the consumers to change their eating habits (Carpentieri et al., 2022). Plant-based ingredients are frequently used in meat products to reduce cost, to improve their techno-functional properties as well as to give added value to those products (Sirini et al., 2022). These ingredients include vegetables proteins, prebiotics, dietary fibers, probiotics, spices and herbs, wich exhibit several health-beneficial physiological effects (Gullón et al., 2020). In particular, dietary fiber has been linked to potential health benefits, preventing the risk of coronary heart diseases, constipation, diabetes, obesity, and some types of cancer (Aslam et al., 2014; He et al., 2022).

Pequi (*Caryocar brasiliense Camb*.), belonging to family Cariocaceae, also known as piqui or piquiá, is a native fruit from Brazilian Cerrado with a significant economic relevance for the local communities (Cornelio-Santiago et al., 2022). It is composed of a large greenish-brown exocarp, white external mesocarp, and internal mesocarp of yellow pulp, which is mostly used in cooking in the typical regional dishes or consumed in the processed forms of creams, liquor, cand, biscuit and jellies, among other by-products (Guedes et al., 2017; Silva et al., 2020). Pequi is a highly nutritious and healthy food with appreciable amounts of insoluble fibers, minerals such as magnesium, zinc, iron and phosphorus, biologically active compounds including phenolics, tocopherols, phytosterols and carotenoids, such as beta-carotene, a precursor to vitamin A (Silva et al., 2022; Torres et al., 2016). Moreover, pequi is also a rich source of unsaturated fatty acids, predominantly oleic (60.6%), which plays a crucial role in hormone synthesis (Pinto et al., 2018).

Recently, numerous studies have reported the biological activities of pequi pulp and oil including anticarcinogenic effects (Brito et al., 2022), antioxidant and anti-inflammatory properties (Torres et al., 2016), analgesic, healing an gastroprotective (de Lacerda Neto et al., 2017) and antibacterial activities (Pereira et al., 2020). Therefore, pequi pulp flour can be used as potentially functional food ingredient in the food system.

Cooking is essential heating process usually applied to meat and some meat products to allow their consumption. Cooking can improve the taste, flavor, tenderness, digestibility, as well as the shelf life of meat products by inactivation of pathogenic microorganisms (Rasinska et al., 2019; Trevisan et al., 2016). On the other hand, cooking may lead to decrease the nutritional value of meat due to protein denaturation, loss of some minerals, vitamins, and water. In addition, cooking can promote the lipid oxidation resulting in the alteration of fatty acid profile (Campo et al., 2013; Lopes et al., 2015). These changes in nutritional value are affected by cooking method, temperature, time and cooking environment (Song et al., 2017).

As a natural functional ingredient, pequi pulp has been reported to improve the quality of several food products such as chicken burgers (Jorge et al., 2022), dark chocolate (Lorenzo et al., 2022), fish (Pereira et al., 2021) and broiler meat (Frasao et al., 2018). However, to the best of our knowledge, there is only one study regarding the use of pequi pulp flour in chicken nuggets (Braga-Souto et al., 2020), but no information is available about the effect of different concentrations of pequi pulp flour and cooking methods on the quality characteristics of chicken nuggets. Therefore, the present study aimed to investigate the effect of pequi flour (PF)

incorporation and different cooking methods such as dee-frying, air-frying, oven-roasting and microwave on the carotenoids content, fatty acid composition, volatile profile and sensory characteristics of chicken nuggets.

2. Material and methods

2.1. Materials

Frozen pequi fruit (*Caryocar brasiliense*) without the exocarp (a greenish-brown outer skin) and the white external mesocarp, harvested in 2021, were obtained from two Fruit Pulp Industries-Barra do Garças (Mato Grosso State, Brazil) and Goiânia (Goias State, Brazil). The fruits were then transported to the Pilot Plant in the Laboratory of Post-Harvest of Fruit and Vegetables Fruit of Federal University of Lavras (UFLA, Lavras, Brazil), where the experiment was carried out. All the ingredients, including the chicken breast fillet were obtained from local markets in Lavras, Brazil.

2.2. Preparation of pequi flour (PF)

Fruits consisted of the internal mesocarp (yellowish-orange pulp) and seeds, without any physical injury and uniform in size and color were visually selected, sanitized with sodium hypochlorite solution (100 mg/L) for 15 min and then rinsed in distilled water to remove the residual chlorine. Afterward, they were bleached in a water bath at 80 ^{o}C for 8 min. After 10 min cooling over in an ice bath, the fruits were then manually pulped using the stainless knife and the pulp and seeds were removed. Subsequently, the pulp was dried in an oven with forced air circulation (Solab SL 102 model, Piracicaba, São Paulo, Brazil) at 40^{o}C for 30 h. The dried pulp was first crushed using an industrial blender (Poli model LS-06, Brusque/Brazil), and then in a knife mill (Tecnal T650 model, Piracicaba, Brazil) and passed through a 20 – mesh sieve (0,85mm) to obtain the PF. The resulted PF was vacuum-packed in polyethylene plastic bags containing approximately 500g and stored frozen at -20 ^{o}C until further use.

2.3. Preparation of chicken nuggets

Five different formulations (Table 1) in three separated batches (repetitions) were prepared in the pilot plant of Laboratory of Meat Science and Technology (LabCarnes) at Federal University of Lavras. Briefly, frozen chicken breast fillets were thawed at 4 ^{o}C overnight, cut into smaller chunks and ground twice in an electric meat grinder (BECCARO model PB-22, Rio Claro, Brazil), first with an 8 mm disc and then with 6 mm disc and

homogenized with all the other ingredients. The final mixture was molded in a hamburger mold (11 cm diameter), immediately frozen at -18 ºC for 8h, cut into four equal pieces (2.5 x 2.5 x 1.0 cm) and refrozen for 1h. After this, the formulated nuggets were predusted with wheat flour, batter-coated with a mixture comprised of wheat flour (16.5%), corn starch (10.0%), powdered milk (6.5%), salt (1%) and ice water (66.0%), and breaded with dried bread crumbs. After coating, the chicken nuggets were pre-fried in soybean oil at 90 ºC for 30 s, immediately removed from the fryer and drained on absorbent papers at room temperature for 10min to remove the excess surface oil. Finally, the chicken nuggets samples were stored in polyethylene plastic bags and frozen at -18 ºC for 45 days. The physicochemical analyses were carried out at 0, 15, 30 and 45 days of frozen time and the sensory evaluations were performed only at first day of storage period.

Table 1 Formulations of the chicken nuggets

Ingredients (%)	Treatments				
	PF0%	PF3%	PF6%	PF9%	PF12%
Chicken breast fillet	81.0	78.0	75.0	72.0	69.0
Pequi flour	0.0	3.0	6.0	9.0	12.0
Water	15.0	15.0	15.0	15.0	15.0
Salt (NaCl)	1.0	1.0	1.0	1.0	1.0
Phosphate	0.5	0.5	0.5	0.5	0.5
Textured soy protein	1.4	1.4	1.4	1.4	1.4
Sodium erythorbate	0.3	0.3	0.3	0.3	0.3
Garlic powder	0.3	0.3	0.3	0.3	0.3
Onion powder	0.3	0.3	0.3	0.3	0.3
White pepper powder	0.1	0.1	0.1	0.1	0.1
Nutmeg powder	0.1	0.1	0.1	0.1	0.1

PF0%, chicken nuggets without pequi flour (control); PF3%, PF6%, PF9% and PF12%, chicken nuggets with 3, 6, 9 and 12% pequi flour, respectively.

2.4. Cooking methods of chicken nuggets

Frozen pre-fried raw chicken nuggets samples were thawed (1h) at room temperature and exposed to different heat treatments as follow: (1) Deep frying, the chicken nuggets were fried using 2.5 L soybean oil in a pre-heated domestic deep fryer at 180 ºC for 4 min (the samples were turned over every minute); (2) Air-frying, the samples were placed into a pre-heated air-fryer (Mondial model AF-34, Brazil) at 180 ºC and air-fried for 15 min; (3) Oven-

roasting, chicken nuggets were roasted in a pre-heated commercial oven at 150 ºC for 8 min; and (4) Microwave, the chicken nuggets samples were placed into a microwave (Brastemap ative, model BMS45B, Brazil) container and cooked at 50% power (410W) for 4 min. The heat temperatures and cooking times were chosen based on the preliminary experiments. After cooking, all samples were cooled down to the room temperature and used for the analyses.

2.5. Experimental design

Data from cooking yield and total carotenoids were arranged in a 5 x 4 factorial design, with five levels of pequi flour (0%, 3%, 6%, 9%, 12%) and four levels of cooking methods (air-frying, deep-frying, oven roasting, and microwave). Data from fatty acid profile and volatile compounds were evaluated using a 3 x 4 factorial arrangement, with three levels of PF (0%, 6%, 12%) and the four levels of cooking methods. The sensory data were assessed using completely randomized block design, including assessors as a random effect (repetitions).

2.6. Analyses

2.6.1. Cooking yield

The cooking yield was estimated by the difference between the cooking nuggets weight and the raw nuggets multiplied by 100 according to equation (1).

$$\text{Cooking yield (\%)} = \left(\frac{\text{Weight after cooking - Weight before cooking}}{\text{Weight after cooking}} \right) \text{x } 100 \quad (1)$$

2.6.2. Extraction and quantification of carotenoids

Carotenoids from cooked chicken nuggets were extracted and quantified based on the procedure described by Rodriguez-Amaya (2001) with minor adjustments. In brief, 2.5g of the chicken nuggets samples were homogenized with 20 mL of cold acetone and vigorous shaking on a shaker (Nova ethics model 109-2TCM, Vargem Grande Paulista, Brazil) for 20 min. The residue was separated from the liquid phase by filtration using a filter paper with 14-μm porosity and washed three times with acetone (20 mL). The filtrate was transferred into a 250 mL separatory funnel to wich 30 mL of petroleum ether and 100 mL of water were added. After phase separation, the lower phase containing acetone and water was discarded and the washing process was repeated three time. The resulting total petroleum ether layer was filtered using a

filter paper with 14-μm porosity and the volume was completed to 50 mL with petroleum ether. The absorbances were measured a UV-VIS spectrophotometer at 444, 450, 456, 462, and 470 nm for α-carotene, β-carotene, δ-carotene, γ-carotene, and lycopene, respectively. The results were expressed in μg/100g of the total carotenoids content using the following equation (2).

$$C = \frac{OD \times V \times 10^6}{A^{1cm1\%} \times W} \times 100 \quad (2)$$

Where, C is the total carotenoid content (μg/100g); OD is the absorbance at each specific wavelength (444, 450, 456, 462, and 470nm); V is the total volume of sample extract solution (mL); $A^{1cm1\%}$ is the specific absorption coefficient of particular carotenoids (α-carotene, β-carotene, δ-carotene, γ-carotene, and lycopene); W is the mass of the sample (g).

2.6.3. Fatty acids profile analysis

The total fat extraction was based on the procedure describer by (Bligh & Dyer, 1959). Briefly, 4g of chicken nuggets (raw, air-frying, deep-frying and oven-roasting) were extracted using methanol, chloroform and water (1:1:2:0.8, v/v/v) mixture and determined by gravimetry in an oven with forced air circulation (Solab SL 102 model, Piracicaba, São Paulo, Brazil) at 105 ºC. Then, 20 mg of fatty acid were converted to fatty acid methyl esters (FAME) by the derivatization reaction proposed by (Hartman & Lago, 1973). The lipid fraction was added 1mL of methanolic potassium hydroxide solution (0.4 M) and the whole was subjected to a boiling point water bath for 10 min. After cooling, 3 mL of methanolic sulfuric acid solution (1 M) was added and again subjected to heating for 10 min. Finally, 2 mL of hexane were added to solubilize the FAME.

The hexane-diluted FAMEs were quantified by gas chromatography (Shimadzu GC 2010) equipped with a fused silica capillary column (Supelco SP-2560, Bellefonte, PA, EUA; 100 m x 0.25 mm x 0.25 μm film thickness), flame ionization detector and injector split ratio was 1:50. The injector and detector temperatures were 260 ºC. Helium was used as the carrier at a constant flow of 0.8mL/min. The operating column conditions were the following: initial temperature of 140°C/5 min, which increased to 240 ºC at a rate of 4°C/min and it remained at this temperature for 30 min for a total run time of 60 min. The fatty acids peaks were identified by comparison with standards available FAME mixtures (37-component FAME Mix; Supelco Inc., Bellefonte, PA, USA) and the results were expressed as percentage of the total detected

FA methyl esters (FAME). The atherogenicity index (AI) and thrombogenicity index (TI) were determined according to equations (3) and (4), developed by Ulbricht & Southgate (1991).

$$AI = (C12:0 + 4 \times C14:0 + C16:0) / \left(\sum MUFA + \sum n\text{-}6 + \sum n\text{-}3\right) \quad (3)$$

$$TI = (C14:0 + C16:0 + C18:0) / \left[\left(0.5 \times \sum MUFA\right) + \left(0.5 \times \sum n\text{-}6\right) + \left(3 \times \sum n\text{-}3\right) + \left(\sum n\text{-}3 / \sum n\text{-}6\right)\right] \quad (4)$$

2.6.4. Volatile compounds analysis

The analysis of volatile compounds from all treatments was performed using the headspace-solid phase microextraction (HS-SPME) technique as previously described by Gomide et al. (2022), with small modifications. Briefly, 5 g of chicken nuggets were placed in a 10 mL sealed glass vial with a PTFE-silicone septum (Supelco, Bellefonte, PA, USA). Divinylbenzene/Carboxen/Polydimethylsiloxane (50-30µm coating thickness; 1 cm length; Supelco, Bellefonte, PA, USA) fiber was used for the extraction of volatile compounds. Prior to extraction, the fiber was conditioned at 220 ^{o}C for 5 min according to manufacturer instructions. The samples were incubated at 40 ^{o}C for 10 min and then extracted at 40 ^{o}C for 30 min, with a constant desorption of 2 min. After extraction, the volatile compounds were separated and identified using a GC-MS QP2010 plus (Shimadzu Corporation, Japan) equipped with an AOC-5000 automatic injector for liquids and gases (Shimadzu, Japan) and an SLBTM column (30 m x 0.25 mm x 0.25 µm 5% phenyl, 95% dimethylsiloxane film. The CG conditions were as follows: the injector temperature was maintained at 220 ^{o}C under splitless mode; high purity helium (99.999%) was used as carrier gas, and the flow rate was kept constant at 1.0 mL/min. The initial oven temperature was set at 40 ^{o}C for 4 min, then increased to 80 ^{o}C at a rate of 2 ^{o}C/min. After, the temperature was increased from 80 ^{o}C to 140 ^{o}C at a rate of 4 ^{o}C/min, and finally increased further for 200 ^{o}C at a rate of 8 ^{o}C/min, where it was maintained until the end. The mass spectrometer was operated under electron impact mode (70 eV) with a full scan mode from 45 to 60 Da and solvent cut-off in 0.55 min. The interface and ion source temperatures were 240 ^{o}C and 200 ^{o}C, respectively. Volatile compounds identification was made by comparing their mass spectra with those of the database (NIST 8, Willey 8 and FFNSC 12) and comparing the retention indices (RI) obtained from the retention time (RT) of homologous series of n-alkanes (C8-C20) with the available literature data. The volatile compounds were reported as relative peak areas (peak area of each compound/total area) x 100.

2.6.5. Sensory analysis

Prior to sensory analysis, the research was approved by the Human Research Ethics Committee of the Federal University of Lavras (CAAE: 55582822.6.0000.5148) and all panelists signed a free and informed consent form, agreeing voluntarily to participate in the study. The volunteers among undergraduate and post-graduate students, staff and professors of the Federal University of Lavras were selected on the basis of their interest, availability and habit in consuming chicken nuggets, at least once a month. The acceptance test using a structured 9-point hedonic scale (1 = dislike very much; 9 = like very much) was applied to assess the effect of different cooking methods on the acceptance of the chicken nuggets. The test was done by a focus group consisted of 19 panelists of both genders, ages between 18 and 50 years, which evaluated the samples in terms appearance, aroma, taste, texture, and overall acceptance. Only the control chicken nuggets samples (without PF) were subjected to four thermal processing methods, including air-frying, deep-frying, oven-roasting and microwaving under conditions described in the section 2.4. After cooking, the samples were coded with random three digits numbers and served to consumers in a monadic order. Room temperature water was provided to panelists for palate cleansing.

2.7. Statistical analysis

Analysis of variance was performed using R version 4.0.4 (R Core Team, 2020). Significant differences between means cooking loss, total carotenoids contents and sensory attributes were detected by Tukey's test. For volatile compounds and fatty acids profile differences among mean were tested by Duncan's multiple range tests. The PCA and hierarchical cluster analysis were carried out using Origin Pro 8.0 (OriginLab, USA). Significant differences were fixed at 5% level.

3. Results and discussion

3.1. Cooking yield and total carotenoids contents

As shown in Fig. 1A and B, the cooking yield and carotenoids content of all samples were affected by both PF concentrations and cooking method ($p < 0.05$). Consistently, the increase of PF in the chicken nugget, resulted in a significant increase of cooking yield, regardless of the cooking method. Analogous observations have been found by (Zaini et al., 2021) and (Verma et al., 2022) in meat nuggets and chicken nuggets formulated with the addition of green peas powder and *Nelumbo nucifera* powder, respectively. The improved

cooking yield might be related to the influence of dietary fiber present in the PF, which probably lead to enhance the water holding capacity (WHC) and fat retention of chicken nuggets (Han & Bertram, 2017). The role of dietary fiber in the improvement of WHC and fat retention in meat products have been demonstrated in numerous studies (Barros et al., 2018; Polizer et al., 2015; Zaini et al., 2021). Barros et al. (2018) observed that the addition of chia flour in chicken nuggets enhanced the water retention and absorption capacity, thus increasing cooking yield as a higher amount of chia flour was added into the formulations. In addition, Zaini et al. (2021) reported a low cooking loss with the increase of green peas powder in chicken nuggets.

With regard to the cooking methods, in general it was observed that air-frying showed higher cooking yield (from 80.27 to 91.60%), followed by oven-roasting (from 82.51 to 90.57%), as the level of PF increased. The higher cooking yield observed in chicken nuggets cooked by air-frying and oven-roasting methods might be related to the formation of harder and denser superficial crust around the chicken nuggets which created a physical barrier that helped to retain the water inside the samples (Echegaray et al., 2020; Yang et al., 2019).

On the other hand, the lower cooking yield observed in microwaved samples could be attributed to the fact that no superficial crust is formed during microwave cooking (Serrano et al., 2007). Moreover, the heat generated during cooking, the high electromagnetic field, the high power and the short exposure time cause myofibrillar proteins denaturation, leading to loss water, and ultimately decreasing of water holding capacity (Echegaray et al., 2020; Wan et al., 2019). Although deep-frying also generates a superficial crust, the higher cooking loss found in this method could be explained by the higher temperature used (180 ^{o}C) in comparison with oven-roasting temperature (150 ^{o}C). Overall, our outcomes are in harmony with those reported by other authors who also observed that microwave cooking and deep-frying resulted in the higher cooking loss in meat products, when compared to other cooking methods (Domínguez et al., 2014; Echegaray et al., 2020; Pathare & Roskilly, 2016).

As expected, due to the higher content of carotenoids in pequi fruit, the inclusion of PF, regardless the cooking method, resulted in significant increase ($p < 0.05$) of the total carotenoids content, showing the highest values at the concentration level of 12% of PF (Fig. 1B), with air-fried samples having the highest total carotenoids content (78.38 µg/100g), followed by microwaved (74.28 µg/100g) and oven-roasted chicken nuggets (72.14 µg/100g). These differences were excepted since carotenoids are very sensitive to heat and cooking treatment mainly depends on the method of cooking, length of heating time, and temperature. On the

other hand, the lowest total carotenoids content observed in deep-fried samples (59.98 µg/100g) might be related to their liposolubility and thermal sensitivity, when treated with heat which were easily dissolved into oil followed by degradation at high temperature (Zhao et al., 2019).

Carotenoids are lipid-soluble pigments widely distributed in plants, algae, fungi, bacteria, and some animals like salmon, shrimp, and crab. These compounds are responsible for the colors from yellow to red in several plant-based sources and fungi (Meléndez-Martínez et al., 2022; Rodriguez-Amaya, 2019). Besides, several studies have demonstrated that the consumption of carotenoid-rich food might lower the risk of some specific diseases including diabetic retinopathy, cardiovascular diseases, bacterial infections, as well as breast, prostate, and skin cancer (Maghsoudi et al., 2022).

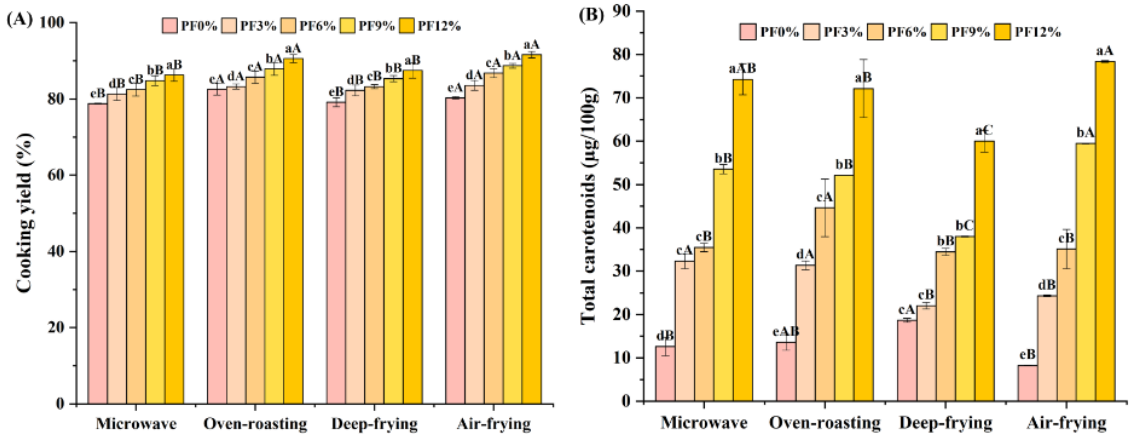


Fig.1. Effects of different levels of pequi flour and cooking methods on cooking yield (A) and total carotenoids (B). PF: pequi flour; PF0%: control chicken nuggets with a traditional formula; PF3%, PF6%, PF9% and PF12%: samples with 3, 6, 9 and 12% pequi flour incorporation, respectively. Different letters (A-C) indicate significant differences between different levels of pequi flour (bars with different colors) for the same cooking method ($p < 0.05$), while different letters (a-c) indicate significant differences between different cooking methods for the same level of PF (bars with the same color) ($p < 0.05$) by Tukey test.

3.2. Fatty acid profile.

The individual fatty acid constituents detected in raw and cooked samples with different methods are shown in Table 2. A total of 25 fatty acids were identified in all treatments, including 9 saturated fatty acids (SFA), 7 monounsaturated fatty acids (MUFA) and 9 polyunsaturated fatty acids (PUFA). Oleic (C18:1n-9c), linoleic (C18:2n-6c), palmitic (C16:0) and stearic (C18:0) fatty acids of the MUFA, PUFA and SFA, respectively, were the most predominant in raw and cooked chicken nuggets. Fatty acid profile was affected by both incorporation of PF and by the effect of cooking method, showing an interaction between them

($p < 0.05$). In raw chicken nuggets, as expected, increasing the proportion of PF significantly increased the content of oleic (C18:1n-9c) and palmitic (C16:0) (fatty acids and decreased the content of linoleic (C18:2n-6c) and stearic (C18:0) fatty acids ($p < 0.05$).

On the other hand, the levels of SFA and MUFA in raw chicken nuggets (Table 3) were found to increase, while the content of PUFA was greatly reduced ($p < 0.05$) with the increase of PF. The increase in the amount of MUFA and SFA was mainly related to the increased levels of oleic and palmitic acids, respectively. The findings are in agreement with the data of previous study by Nascimento-Silva & Naves (2019) who reported a significant content of oleic and palmitic fatty acids in pequi pulp. On the other hand, the decrease in the content of PUFA was mainly due to the decrease content of individual PUFAs, mainly linoleic acid (C18:2n-6c).

The ΣPUFA/ΣSFA, n-6/n-3, Atherogenicity index (AI) and thrombogenicity index (TI) ratio indices are the most important parameters used to evaluate the nutritional value and the healthiness of meat products for human consumption (de Souza Paglarini et al., 2019; Wereńska et al., 2021). In the present study, ΣPUFA/ΣSFA ratio in raw chicken nuggets decreased with increasing levels of PF, due to their high SFAs content (Table 3). This outcome suggested that increasing the PF in raw chicken nuggets up to 12% was not effective in achieving the recommended ΣPUFA/ΣSFA ratio (> 0.4) for balanced diet (FAO/WHO, 2010).

As regard the n-6/n-3 ratio (Table 3), an increase in the concentration of PF in raw chicken nuggets resulted in the desirable decrease of this ratio in range from 8.23-6.64%. However, the ratio remained high, since the recommended n-6/n-3 value for a healthy profile is < 4 (Salcedo-Sandoval et al., 2014). High n-6/n-3 ratio may lead to lipogenesis and hepatic steatosis, thus increasing the risk of cardiovascular disease and adverse coronary events (Valenzuela & Videla, 2011). Besides, high levels of n-6 fatty acids are linked with undesirable alterations in lipid composition, such as increased total cholesterol, LDL, and triglycerides (Liao et al., 2020).

The incorporation of PF into the raw chicken nuggets significantly ($p < 0.05$) increased the AI and TI indexes (Table 3). Despite this increase, it is important to highlight that the values of IA and IT reported in this study are within the acceptable limit for a good healthy profile meat product (< 1.0) (Di Bella et al., 2022). A lower value of these indexes is linked with a higher good fatty acid quality that help to lower the risk of coronary diseases (Saygi et al., 2018).

In reference to cooking methods, in general, oven-roasting resulted in a significant ($p < 0.05$) higher content of SFA in comparison with the other cooking methods, with deep-frying having a significantly lower content, while no significant differences were observed among air-fried, microwaved, and raw samples, which suggest that these methods had less impact on SFA (Table 4). In this study, the significant increase of SFA levels in oven-roasted samples as compared with deep-fried, air-fried, microwaved and raw samples might be related to highest palmitic (C16:0) content in oven-roasted, which is responsible for formation of SFAs. Likewise, (Yu et al., 2021) reported a significant increase of SFAs content in roasted Piao chicken breast meat.

MUFA content decreased significantly ($p < 0.05$) after cooking regardless the formulation or the cooking method. MUFA levels were higher in microwaved followed by air-fried, deep-fried and oven-roasted chicken nuggets samples. The decrease of MUFA content might be related to the decrease of the oleic acid (C18:1n-9c), the predominant monounsaturated fatty acid in PF, which undergoes oxidative degradation to form aldehydes, including heptanal and octanal, during processing (Zang et al., 2020). On the other hand, the PUFA content in all cooking methods was significantly higher when compared with the raw samples. The PUFA levels in deep-fried samples was significantly higher than of air-fried, microwaved and oven roasting. The lower levels of PUFAs in oven roasting samples was explained by a significant decrease in the proportion of individual PUFAs, mainly linoleic acid.

Deep-frying cooking process of chicken nuggets, in the present study, resulted in the highest ($p < 0.05$) ΣPUFA/ΣSFA ratio when compared with other cooking methods, which did not differ ($p > 0.05$) from the raw samples. The higher ratio in deep-fried samples is possibly due to the significantly higher content of linoleic acid (C18:2n6c). In this study it was observed that in all cooking methods, the formulations with 6% of pequi remained higher ΣPUFA/ΣSFA ratios than 0.4 as recommended by World Health Organization (WHO/FAO, 2003).

All cooking methods in the present study resulted in higher n-6/n-3 ratios than the minimum recommended values for a good quality meat product (< 4). The n-6/n-3 ratio was significantly lower in microwaved samples, whereas the oven-roasted samples had the highest ratio when compared to other cooking methods and raw samples.

Regarding AI, no significant differences were observed between air-fried and microwaved chicken nuggets in comparison with the raw samples, whereas deep-frying caused a significant decrease in this parameter (Table 3). On the other hand, oven-roasting resulted in

a slight increase in the IA values when compared to raw samples. Despite these changes, all cooking methods retained the AI index within the acceptable limit (< 1.0).

All the cooking methods, in general, resulted in a decrease of TI with samples cooked by oven-roasting having a significantly lower value, whereas microwaved samples presented the higher TI index values, followed by air-fried and deep-fried chicken nuggets, when compared to raw samples (Table 3).

Table 2 Effects of different levels of pequi flour (PF) and different cooking methods on the fatty acids profile of chicken nuggets

Cooking methods	Raw			Air-frying			Deep-frying			Oven-roasting			Microwave		
Pequi flour (%)	PF0%	PF6%	PF12%	PF0%	PF6%	PF12%	PF0%	PF6%	PF12%	PF0%	PF6%	PF12%	PF0%	PF6%	PF12%
Fatty acid composition (%)															
C12:0	/	0.06^{abA}	0.06^{aA}	0.08^{aA}	0.07^{aAB}	0.06^{aB}	0.04^{bA}	0.04^{bA}	0.05^{aA}	0.07^{aA}	0.06^{abAB}	0.05^{aB}	0.08^{aA}	0.04^{bB}	0.06^{aB}
C14:0	0.66^{bA}	0.42^{abB}	0.32^{aC}	0.79^{aA}	0.47^{aB}	0.34^{aC}	0.69^{bA}	0.37^{bB}	0.39^{aB}	0.80^{aA}	0.38^{bB}	0.29^{aB}	0.77^{aA}	0.35^{bB}	0.39^{aB}
C14:1	0.09^{aA}	0.05^{bB}	0.03^{aB}	0.11^{aA}	0.05^{bB}	0.03^{aB}	0.07^{aA}	0.03^{bB}	0.03^{aB}	0.09^{aB}	0.48^{aA}	0.02^{aC}	0.08^{aA}	/	0.03^{aB}
C15:0	0.06^{aA}	0.04^{aB}	0.03^{aC}	0.07^{aA}	0.04^{aB}	0.03^{aC}	0.09^{aA}	0.04^{aB}	0.03^{aC}	0.08^{aA}	0.03^{aB}	0.03^{aC}	0.08^{aA}	0.04^{aB}	0.03^{aC}
C16:0	21.08^{aB}	29.85^{aA}	30.80^{bA}	20.57^{aC}	28.31^{bB}	31.31^{bA}	20.63^{aC}	24.77^{cB}	27.66^{cA}	19.92^{abC}	29.38^{abB}	37.68^{aA}	18.90^{bC}	25.90^{cB}	31.25^{bA}
C16:1	2.93^{aA}	1.69^{aB}	1.40^{aB}	2.58^{aA}	1.50^{aB}	1.46^{aB}	2.65^{aA}	1.37^{aB}	1.03^{bC}	2.76^{aA}	1.40^{aB}	0.97^{bC}	2.50^{aA}	1.38^{aB}	1.52^{aB}
C17:0	0.14^{aA}	0.11^{aB}	0.08^{abC}	0.11^{aA}	0.09^{abA}	0.09^{aA}	0.12^{aA}	0.11^{aAB}	0.10^{aB}	0.13^{aA}	0.08^{bB}	0.06^{bB}	0.13^{aA}	0.09^{abB}	0.09^{aB}
C17:1	0.10^{cA}	0.07^{bB}	0.05^{aB}	0.21^{aA}	0.11^{aB}	0.06^{aC}	0.19^{aA}	0.08^{abB}	0.06^{aC}	0.16^{bA}	0.06^{bB}	0.05^{aB}	0.15^{bA}	0.08^{abB}	0.04^{aC}
C18:0	6.82^{bA}	4.34^{bB}	3.64^{bC}	6.90^{bA}	4.36^{bB}	3.49^{bC}	7.34^{aA}	4.59^{aB}	3.99^{aC}	6.64^{cA}	3.47^{cB}	2.87^{cC}	6.50^{bA}	3.99^{bB}	3.96^{bC}
C18:1n9t	/	/	/	/	0.06^{bB}	0.11^{aA}	0.09^{aB}	0.11^{bA}	0.10^{aB}	0.08^{aA}	/	0.05^{bB}	0.09^{aB}	0.05^{bC}	0.11^{aA}
C18:1n9c	33.38^{bcC}	48.36^{aB}	52.93^{aA}	32.31^{cC}	47.30^{aB}	50.51^{bA}	33.27^{bcC}	41.51^{bB}	47.32^{cA}	34.58^{abB}	47.22^{aA}	46.31^{cA}	35.90^{aC}	43.20^{bB}	50.29^{bA}
C18:2n6c	30.08^{aA}	12.68^{dB}	7.36^{cC}	30.69^{aA}	14.98^{cB}	10.29^{bC}	29.60^{aA}	23.21^{aB}	16.14^{aC}	30.18^{aA}	16.22^{cB}	9.03^{bC}	28.91^{aA}	18.80^{bB}	9.29^{bC}
C20:0	0.22^{aA}	0.18^{bA}	0.20^{aA}	0.18^{aA}	0.20^{bA}	0.19^{aA}	0.21^{aA}	0.27^{aA}	0.25^{aA}	0.20^{aA}	0.18^{bA}	0.16^{aA}	0.20^{aB}	0.32^{aA}	0.19^{aB}
C18:3n6	/	/	/	/	0.05^{bA}	0.04^{abA}	0.08^{aA}	0.06^{bA}	0.02^{bcB}	0.08^{aA}	0.03^{bcB}	0.02^{bcB}	0.07^{aB}	0.11^{aA}	0.07^{aB}
C20:1	0.23^{aA}	0.21^{aA}	0.21^{aA}	0.16^{bB}	0.21^{aA}	0.23^{aA}	0.26^{aA}	0.17^{aB}	0.20^{aB}	0.21^{aA}	0.18^{aAB}	0.15^{bB}	0.24^{aA}	0.19^{aB}	0.18^{abB}
C18:3n3	2.57^{aA}	1.10^{cdB}	0.71^{bB}	2.19^{abA}	1.33^{cB}	1.06^{abB}	2.21^{abA}	2.19^{bA}	1.53^{aB}	1.99^{bA}	0.76^{dB}	0.68^{bB}	2.72^{aB}	4.12^{aA}	0.81^{bC}
C20:2	0.19^{aA}	0.08^{aB}	0.05^{aB}	0.23^{aA}	0.10^{aB}	0.07^{aB}	0.22^{aA}	0.09^{aB}	0.31^{aB}	0.23^{aA}	0.06^{aB}	0.04^{aB}	0.21^{aA}	0.09^{aB}	0.06^{aB}
C22:0	0.21^{aA}	0.08^{dB}	0.08^{bcB}	0.21^{aA}	0.14^{cB}	0.11^{bB}	0.19^{aB}	0.23^{bA}	0.17^{aB}	0.18^{aA}	0.08^{dB}	0.06^{cB}	0.19^{aB}	0.42^{aA}	0.07^{cC}
C20:3n6	0.25^{cA}	0.15^{aB}	0.10^{aC}	0.36^{aA}	0.16^{aB}	0.10^{aC}	0.31^{bA}	0.14^{aB}	0.10^{aC}	0.31^{bA}	0.10^{bB}	0.08^{aB}	0.32^{bA}	0.15^{aB}	0.10^{aC}
C22:1n9	0.13^{aA}	/	/	/	/	/	0.05^{bA}	/	/	/	/	/	/	/	/
C20:3n3	1.43^{abcA}	0.77^{abcB}	0.45^{abcC}	1.67^{aA}	0.81^{aB}	0.52^{aC}	1.45^{bcA}	0.68^{bcB}	0.43^{bcC}	1.52^{cA}	0.46^{cB}	0.37^{cC}	1.55^{abA}	0.73^{abB}	0.49^{abC}
C20:4n6	/	/	/	0.13^{aA}	0.09^{aB}	0.05^{aC}	0.06^{bA}	0.06^{bA}	0.04^{aB}	0.04^{cA}	/	/	/	/	0.05^{aA}
C22:2	0.16^{aA}	0.10^{cB}	0.12^{aB}	/	/	/	0.11^{cA}	0.13^{bA}	0.12^{aA}	0.08^{dA}	0.09^{cA}	0.08^{bA}	0.14^{bB}	0.22^{aA}	0.10^{abC}
C24:0	/	/	/	/	0.07^{aB}	0.08^{aA}	/	/	/	/	/	/	/	/	/
C22:6n3	0.11^{cA}	0.05^{bB}	0.05^{aB}	0.13^{abA}	0.08^{aB}	0.04^{aC}	0.14^{aA}	0.06^{bB}	0.04^{aC}	0.12^{bcA}	0.04^{cB}	0.04^{aB}	0.11^{cA}	0.06^{bB}	0.04^{aC}

Data were presented as mean of three replicates (n = 3). PF: pequi flour; PF0%: control chicken nuggets with a traditional formula; PF6% and PF12%: samples with 6 and 12% pequi flour incorporation, respectively. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; AI: atherogenicity index; TI: thrombogenicity index. Different letters (A-C) indicate significant differences between different levels of pequi flour for the same cooking method ($p < 0.05$), while different letters (a-e) indicate significant differences between different cooking methods for the same level of pequi flour ($p < 0.05$) by Duncan's test. " / "- not detected

Table 3 Effects of different levels of pequi flour (PF) and different cooking methods on fatty acids groups sum, ratios and health indexes of chicken nuggets

Cooking methods	Raw			Air-frying			Deep-frying			Oven-roasting			Microwave		
Pequi flour (%)	PF0%	PF6%	PF12%	PF0%	PF6%	PF12%	PF0%	PF6%	PF12%	PF0%	PF6%	PF12%	PF0%	PF6%	PF12%
Total															
Σ SFA	28.51^{aC}	34.26^{aB}	35.95^{bA}	28.56^{aC}	33.70^{aB}	35.36^{bA}	28.80^{aB}	29.94^{bB}	32.36^{cA}	28.38^{aC}	34.11^{aB}	41.64^{aA}	27.83^{aC}	30.21^{bB}	36.61^{bA}
Σ MUFA	37.32^{abcC}	50.45^{aB}	54.66^{aA}	35.43^{cC}	49.03^{abB}	51.66^{bA}	36.61^{bcC}	42.52^{dB}	48.26^{cA}	38.01^{abB}	48.41^{bA}	48.10^{cA}	38.96^{aC}	45.26^{cB}	52.43^{bA}
Σ PUFA	34.23^{aA}	14.78^{dB}	8.78^{dC}	34.77^{aA}	16.60^{cB}	12.26^{bC}	34.03^{aA}	26.82^{aB}	17.96^{aC}	34.11^{aA}	17.67^{cB}	10.17^{cdC}	34.08^{aA}	23.95^{bB}	11.20^{bcC}
Σ n-3	3.76^{aA}	1.78^{cB}	1.17^{bC}	3.96^{aA}	2.52^{bB}	1.49^{aC}	3.98^{aA}	2.82^{bB}	2.05^{bC}	3.85^{aA}	1.40^{cB}	1.04^{bB}	3.79^{aB}	4.82^{aA}	1.40^{bC}
Σ n-6	30.24^{bA}	12.73^{dB}	7.61^{cC}	33.77^{aA}	14.33^{cdB}	10.53^{bC}	29.98^{bA}	23.35^{aB}	15.93^{aC}	29.71^{bA}	16.46^{cB}	8.97^{bcC}	29.61^{bA}	19.25^{bB}	9.78^{bcC}
Ratio															
ΣPUFA/ΣSFA	1.33^{aA}	0.41^{bB}	0.27^{bB}	1.26^{aA}	0.54^{bB}	0.37^{bB}	1.33^{aA}	0.84^{aB}	0.58^{aC}	1.32^{aA}	0.52^{bB}	0.25^{bC}	1.24^{aA}	0.74^{aB}	0.33^{bC}
n-6/n-3	8.23^{aA}	7.03^{cB}	6.64^{bcB}	7.23^{aA}	6.16^{cB}	6.30^{cB}	7.13^{aA}	7.98^{bA}	8.01^{aA}	7.81^{aB}	12.96^{aA}	8.29^{aB}	7.83^{aA}	4.00^{dB}	7.47^{abA}
n-3/n-6	0.13^{aA}	0.14^{bA}	0.15^{abA}	0.12^{aB}	0.14^{bAB}	0.16^{aA}	0.14^{aA}	0.13^{bA}	0.13^{bA}	0.13^{aA}	0.08^{cB}	0.13^{bA}	0.14^{aB}	0.25^{aA}	0.15^{aB}
Indixes															
AI	0.34^{aC}	0.46^{aB}	0.50^{bA}	0.32^{aB}	0.47^{aA}	0.49^{bA}	0.31^{aB}	0.39^{bA}	0.43^{cA}	0.34^{aC}	0.48^{aB}	0.70^{aA}	0.32^{aC}	0.40^{bB}	0.51^{bA}
TI	0.63^{aC}	0.91^{aB}	1.04^{bA}	0.59^{aC}	0.80^{bB}	0.93^{cA}	0.68^{aB}	0.75^{bAB}	0.82^{dA}	0.62^{aC}	0.92^{aB}	1.25^{aA}	0.59^{aB}	0.62^{cB}	1.02^{bcA}

Data were presented as mean of three replicates (n = 3). PF: pequi flour; PF0%: control chicken nuggets with a traditional formula; PF6% and PF12%: samples with 6 and 12% pequi flour incorporation, respectively. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; AI: atherogenicity index; TI: thrombogenicity index. Different letters (A-C) indicate significant differences between different levels of pequi flour for the same cooking method ($p < 0.05$), while different letters (a-e) indicate significant differences between different cooking methods for the same level of pequi flour ($p < 0.05$) by Duncan's test. " / "- not detected

3.3. Volatile compounds

Volatile compounds are critically responsible for the creation of aroma in food matrices and play a pivotal role in the food acceptance by consumers (Cheseto et al., 2020). A total of 45 substances including 13 esters, 4 alcohols, 15 terpenes, 5 aldehydes, 3 ketones, aliphatic hydrocarbons, and 1 aromatic hydrocarbon were detected across all sample treatments (Table 4). In raw chicken nuggets, the top two majority groups were terpenes and esters, which were followed by the group of alcohols, aldehydes, ketones, aliphatic hydrocarbons and aromatic hydrocarbons. The volatile compounds in higher concentration in each group (Z)-β-Ocimene of terpenes, ethyl hexanoate of esters, terpinene-4-ol of alcohols, hexanal of aldehydes and 2-propanone of ketones, respectively.

Incorporation of PF and cooking method resulted in significant differences in the levels of volatile compounds of chicken nuggets ($p < 0.05$). In general, the contents of terpenes and esters in raw chicken nuggets increased significantly ($p < 0.05$) with the increasing levels of PF. The total terpenes concentration increased in range from 25.08 to 52.92%, and the esters concentration increased in range from 18.17 to 47.52%. The increase of terpenes and esters with the increase levels of pequi is due to the significant ($p < 0.05$) increase of (Z)-β-Ocimene (1.29-42.84%) and ethyl hexanoate (5.72-39.15%), respectively, which were the most dominant compounds as commented above. It has been reported that esters were the most abundant volatile components in pequi pulp, being ethyl hexanoate one of the individual esters with the highest content (Belo et al., 2013; Damiani et al., 2009). Moreover, the addition of spices in the formulation might also have contributed to the production of a lot of terpenes, mainly (Z)-β-Ocimene (Yao et al., 2022). On the other hand, the contents of alcohols, aldehydes, ketones and aliphatic hydrocarbons decreased significantly ($p < 0.05$) with the increasing proportions of PF in raw chicken nuggets.

There were significant ($p < 0.05$) differences in the volatile compounds content of the chicken nuggets as a result of different cooking methods, as shown in Table 4. Compared with raw chicken nuggets, the contents of terpenes increased significantly after cooking by different methods, especially in the formulations with 12% of PF. The slight increase of terpenes might be attributed to the high temperatures that promoted the lipid degradation and oxidation. Previous study reported that terpenes, aldehydes, ketones, acids, esters, and furans are mainly generated by degradation and oxidation (Zhang et al., 2023).

Table 4 Effects of different levels of pequi flour (PF) and different cooking methods on the volatile compounds of chicken nuggets

Cooking methods		Raw			Air-frying			Deep-frying			Oven-roasting			Microwave		
Pequi flour (%)		PF0%	PF6%	PF12%	PF0%	PF6%	PF12%	PF0%	PF6%	PF12%	PF0%	PF6%	PF12%	PF0%	PF6%	PF12%
Compounds																
Terpenes	**RI**															
α-pinene	932	/	/	/	3.63bA	0.53aB	0.20aB	3.70bA	0.31aB	0.14aB	3.93abA	0.27aB	0.28aB	4.27aA	0.27aB	0.16aB
α-terpinene	1016	1.48cA	0.36aB	0.17aC	1.86aA	0.36aB	0.14aC	1.54cA	0.19bB	0.10aC	1.76bA	0.18bB	0.18aB	0.73dA	0.15bB	0.09aB
β-phellandrene	971	3.25bA	0.70aB	0.34aB	3.12bA	0.67aB	0.19aB	4.24aA	0.29aB	0.17aB	3.44bA	0.25aB	0.26aB	2.40cA	0.41aB	0.18aB
γ-terpinene	1058	3.46bcA	1.01aB	0.50aC	3.61abA	0.79bB	0.33bC	3.43cA	0.49cB	0.27bC	3.72aA	0.46cB	0.52aB	1.80dA	0.43cB	0.24bC
(E)-β-ocimene	1034	/	3.76aA	3.63bA	/	4.05aA	4.16aA	/	3.93aA	4.02aA	/	3.82aB	4.09aA	/	3.90aB	4.15aA
(Z)-β-ocimene	1045	1.29aC	42.84cA	40.35cB	2.84aB	45.96bA	44.91bA	1.94aC	46.22abA	43.73bB	2.45aC	45.74bA	44.54bB	2.04aB	47.21aA	46.96aA
Phellandrene	1005	1.84aA	0.27aB	0.20aB	1.41cA	0.19aB	0.12aB	1.20dA	0.21aB	0.12aB	1.16dA	0.23aB	0.11aC	1.60bA	/	/
m-cymene	1024	4.70aA	0.94aB	0.41aC	2.30cA	0.34bB	/	2.73bA	0.25bB	0.18aB	2.86bA	0.23bB	0.45aB	2.85bA	0.31bB	/
Limonene	1029	3.11cA	0.65aB	0.43aB	5.70aA	0.85aB	0.49aB	4.96b	0.63a	0.44a	5.00bA	0.66aB	0.48aB	5.56aA	0.49aB	0.31aB
Myrcene	987	/	0.56aA	0.54aA	3.94bA	0.61aB	0.24aB	4.44aA	0.26aB	0.16aB	3.73bA	0.22aB	0.38aB	2.56cA	0.30aB	/
p-mentha-1,3,8-triene	1129	/	/	/	/	0.40bA	0.44bA	/	0.33cB	0.45bA	/	0.40bB	0.46bA	/	0.45aB	0.54aA
α-thujene	925	2.39aA	0.38aB	0.19aC	/	/	/	/	/	/	/	/	/	/	/	/
β-Pinene	977	2.91aA	0.51aB	0.42aC	/	/	/	/	/	/	/	/	/	/	/	/
terpinolene	1139	/	0.64aA	0.59aB	/	/	/	/	/	/	/	/	/	/	/	/
Trans-caryophyllene	1423	0.64aA	0.27aB	0.11aC	/	/	/	/	/	/	/	/	/	/	/	/
Total terpenes		**25.08bC**	**52.92aA**	**47.87cB**	**28.40aC**	**54.75aA**	**51.22abB**	**28.18aC**	**53.13aA**	**49.79bB**	**28.05aB**	**52.48aA**	**51.76aA**	**23.81bB**	**53.92aA**	**52.63aA**
Esters																
Ethyl 2-hexenoate	1042	/	0.95aB	1.05aA	/	0.5dB	0.63cA	/	0.52cdB	0.71bA	/	0.53cB	0.61cA	/	0.62bB	0.69bA
Ethyl 2-methylbutyrate	845	/	/	/	/	0.30bA	0.29bA	/	0.29bA	0.30bA	/	0.25bB	0.38aA	/	0.39aA	0.32abA
Ethyl isovalerate	849	/	/	/	0.51abA	0.51bA	0.47bA	0.29cB	0.50bA	0.46bA	0.44bB	0.43bB	0.6aA	0.57aB	0.79aA	0.59aB
Ethyl octanoate	997	/	/	/	15.14aC	31.96bB	37.96aA	10.87bC	34.19aB	38.63aA	11.08bC	34.24aB	37.91aA	9.70bB	34.81aA	35.95bA
Methyl heptanoate	922	/	/	0.39aA	2.70aA	0.45aB	0.40aB	1.98bA	0.49aB	0.37aB	2.06bA	0.51aB	0.33aB	2.65aA	0.29aB	0.36aB
Isopentyl hexanoate	1247	/	0.83aA	0.94aA	/	0.62bA	0.72bcA	/	0.52bB	0.83abA	/	0.63bA	0.68cA	/	0.63bB	0.91aA
Isopentyl pentanoate	1106	/	/	/	/	0.59abA	0.68aA	/	0.43cB	0.65aA	/	0.56bB	0.66aA	/	0.67aA	0.73aA
Ethyl lactate	756	/	4.47aB	5.01aA	/	/	/	/	/	/	/	/	/	/	/	/
Ethyl butyrate	800	3.77aA	0.44aB	0.30aB	/	/	/	/	/	/	/	/	/	/	/	/
Ethyl hexanoate	996	5.72aC	31.64aB	39.15aA	/	/	/	/	/	/	/	/	/	/	/	/
Cis-sabinene hydrate acetate	1070	2.37aA	0.36aB	0.19aC	/	/	/	/	/	/	/	/	/	/	/	/
Trans-sabinenehydrate	1101	1.71aA	0.26aB	0.14aC	/	/	/	/	/	/	/	/	/	/	/	/
Methyl (E) Cinnamate	1293	4.60aA	0.88aB	0.34aB	/	/	/	/	/	/	/	/	/	/	/	/
Total esters		**18.17aC**	**39.84aB**	**47.52aA**	**18.34aC**	**34.94cB**	**41.15bcA**	**13.13bC**	**36.94bB**	**41.95bA**	**13.56bC**	**37.15bB**	**41.18bcA**	**12.93bB**	**38.20abA**	**39.56cA**

Alcohols																
2-methyl-1-butanol	757	1.07dA	0.40abB	0.49aB	2.61cA	0.28bB	0.21bB	4.61aA	0.45aB	0.26bC	2.94bA	0.42abB	0.14bC	2.74cA	0.53aB	0.28bC
3-methyl-1-butanol	755	/	/	/	6.08bcA	0.60bB	0.51aB	9.38aA	0.98abB	0.67aB	5.71cA	1.27aB	0.44aC	6.38bA	1.04abB	0.64aB
1-hexanol	708	4.08aA	1.35aB	0.95aC	0.79cA	/	/	0.95cA	/	/	0.87cA	/	/	1.18bA	0.13bB	/
Terpinen-4-ol	1181	14.48aA	2.39aB	1.32aB	6.00cA	1.29aB	0.77aB	8.68bA	1.06aB	0.85aB	6.16cA	1.09aB	0.53aB	4.37dA	1.17aB	0.84aB
Total alcohols		**19.63bA**	**4.14aB**	**2.77aB**	**15.47cA**	**2.16aB**	**1.50aB**	**23.62aA**	**2.48aB**	**1.78aB**	**15.69cA**	**2.78aB**	**1.11aC**	**14.66cA**	**2.86aB**	**1.76aB**
Aldehydes																
Benzaldehyde	962	7.84aA	0.14aB	0.14aB	1.46bA	0.64aB	0.64aB	0.89bA	/	0.14aB	1.21bA	0.21aB	0.07aB	/	0.34aA	0.23aA
Butanal	700	/	/	/	4.32bA	1.00aB	0.67aC	4.60aA	0.72bB	0.36bC	4.41abA	0.65bB	0.33bC	1.55cA	0.36cB	0.17bcB
3-methylbutanal	717	1.20bA	/	/	1.20bA	0.36aB	0.21aB	0.95cA	/	/	2.51aA	0.09bB	/	/	/	/
Hexanal	801	10.05aA	/	/	10.40aA	0.87aB	0.52aB	6.80bA	0.10aB	0.62aB	7.79bA	0.25aB	0.47aB	10.22a	1.45a	0.77a
Nonanal	1103	0.60cA	/	/	1.38bA	0.56abB	0.57aB	1.30bA	0.20cC	0.70aB	1.58aA	0.48bB	0.58aB	/	0.65aA	0.62aA
Total aldehydes		**19.69aA**	**0.14cB**	**0.14cB**	**18.75bA**	**3.43aB**	**2.61aB**	**14.53dA**	**1.03bcB**	**1.83abB**	**17.51cA**	**1.68bB**	**1.45bB**	**11.77eA**	**2.80aB**	**1.78abC**
Ketones																
3-decanone	978	2.97aA	1.26aB	0.82aC	/	/	/	/	/	/	/	/	/	/	/	/
1-methoxy-3,3-dimethyl-2-butanone	940	/	/	/	/	0.11cB	0.19bA	/	0.34aB	0.44aA	/	0.26b	0.43a	/	/	0.22bA
2-propanone	684	7.74eA	/	/	12.24dA	3.76aB	2.71aC	13.71cA	4.33aB	3.30aC	18.16bA	4.53aB	3.28aC	33.27aA	1.56bB	1.60bB
Total ketones		**10.72eA**	**1.26bB**	**0.82cB**	**12.24dA**	**3.87aB**	**2.90aC**	**13.71cA**	**4.67aB**	**3.73aC**	**18.16bA**	**4.79aB**	**3.71aC**	**33.27aA**	**1.56bB**	**1.83bB**
Aliphatic hydrocarbons																
Decamethyl-cyclopentasiloxane	1132	4.11aA	1.19aB	0.60aC	3.28bA	0.22bB	0.16bB	0.93dA	0.25bB	0.13bB	1.24cA	0.23bB	0.13bB	1.26cA	0.20bB	0.30bB
Propylcyclopropane	868	/	/	/	/	0.20cA	0.16bcA	2.58aA	1.12aB	0.69aC	1.02bA	0.55bB	0.43abB	/	0.26cA	0.18bcA
Heptane	799	/	/	/	2.99bA	0.32aB	0.18aB	2.16cA	0.21aB	0.10aB	3.97aA	0.22aB	0.24aB	2.30cA	0.20aB	0.19aB
1-chloropentane	777	2.60aA	0.51aB	0.29aC	/	/	/	/	/	/	/	/	/	/	/	/
Total aliphatic hydrocarbons		**6.71aA**	**1.70aB**	**0.89aC**	**6.27bA**	**0.74bB**	**0.50aB**	**5.67cA**	**1.58aB**	**0.92aC**	**6.23bA**	**1.00bB**	**0.79aB**	**3.56dA**	**0.66bB**	**0.67aB**
Aromatic hydrocarbons																
Toluene	776	/	/	/	0.54cA	0.11aB	0.11bB	1.16aA	0.17aB	/	0.79bA	0.12aB	/	/	/	1.76aA
Total aromatic hydrocarbons		/	/	/	**0.54cA**	**0.11aB**	**0.11bB**	**1.16aA**	**0.17aB**	/	**0.79bA**	**0.12aB**	/	/	/	**1.76aA**

Data were presented as mean of three replicates (n = 3). PF: pequi flour; PF0%: control chicken nuggets with a traditional formula; PF6% and PF12%: samples with 6 and 12% pequi flour incorporation, respectively. RI: retention index. Different letters (A-C) indicate significant differences between different levels of pequi flour for the same cooking method ($p < 0.05$), while different letters (a-e) indicate significant differences between different cooking methods for the same level of pequi flour ($p < 0.05$) by Duncan's test. " / "- not detected.

All the four cooking methods resulted in a significant ($p < 0.05$) decrease of the total ester content when compared to the raw samples, with air-fried chicken nuggets having significantly higher ($p < 0.05$) levels of esters than those of the other samples, which did not show significant differences each other. Interestingly, compared with the raw samples it can be observed that during cooking some esters (ethyl hexanoate, ethyl lactate, ethyl butyrate, cis-sabinene hydrate acetate, trans-sabinenehydrate and methyl (E) cinnamate) disappeared while other including ethyl 2-methylbutyrate, ethyl isovalerate, ethyl octanoate and isopentyl pentanoate were newly generated. The decrease in the concentration of total ester during cooking might be related to the intensified reactions caused by chicken nuggets components which might have degraded the heat-sensitive and unstable esters and undergo alcoholysis, ester exchange, ammonolysis, and reductions reactions to form new volatile compounds (Xu et al., 2022).

Overall, the content of alcohol was significantly higher in the raw chicken nuggets than that in the cooked samples ($p < 0.05$), but there were no significant differences among the different cooking methods, indicating that air-frying, deep-frying, oven-roasting and microwaving treatments were able to improve the aroma of raw chicken nuggets. The reduction in the total alcohol content of the cooked samples might be related to degradation of alcohols into corresponding acids, esters, and aldehydes (Sun et al., 2010).

In general, all the cooking methods induced a significant increase ($p < 0.05$) in the aldehyde's contents in comparison with raw samples (Table 4). The contents of total aldehydes were significantly higher in air-fried and oven-roasting chicken nuggets than those of the other samples. The increase in the total aldehyde content of cooked chicken nuggets may be attributed to the lipid oxidation and fatty acid (Watanabe et al., 2015). Previous studies have reported that aldehydes play a major role in the aroma of chicken meat products due to their strong volatility and low odor thresholds, which are mainly generated of unsaturated fatty acids, lipid oxidation and amino acid degradation (Wu & Wang, 2019; Yin et al., 2021). Among the aldehydes detected in the present study, hexanal and benzaldehyde are recognized for imparting rancid flavors to meat products and these are mostly derived from the oxidation of linoleic acid first to 2,4-decadienal, which then continues to degrade (Rasinska et al., 2019; S. Wu et al., 2021). On the other hand, nonanal is usually generated by the oxidative degradation of oleic acid (Watanabe et al., 2015).

Cooking methods also resulted in a significant ($p < 0.05$) increase in the contents of ketones in comparison with raw samples (Table 4). Interestingly, in the formulations without PF the content of total ketones was significantly higher in microwaved chicken nuggets, whereas the air-fried chicken nuggets had the lowest content of ketones among the samples. However, in the samples treated with PF, oven-roasting, air-frying and deep-frying resulted in a significant higher content of ketones than microwave. The increase of total ketones contents is likely related to the accelerated lipid oxidation, Maillard reaction and Strecker degradation by the different cooking methods (Manyatsi et al., 2022). Ketones are also mainly derived from amino acid degradation and unsaturated fatty acid oxidation (Zhu et al., 2019). These results suggested that deep-frying, air-frying, oven-roasting and microwave can improve the overall aroma of chicken nuggets by increasing the content of ketones.

Air-frying, deep-frying, oven-roasting, and microwave treatment significantly decreased the content of aliphatic hydrocarbons and increased the content of aromatic hydrocarbons compared to raw chicken nuggets. The total content of aliphatic hydrocarbons was lower in the microwaved samples than those of the other samples ($p < 0.05$), suggesting that microwaving had a lower impact on the lipid degradation. Aliphatic and aromatic hydrocarbons are also well-known as indicator of lipid oxidation that can play a critical role in the formation of the pleasant flavor in meat product, in addition to off-flavor and toxic compounds at high temperatures (Toldrá & Reig, 2012).

3.3.1. Hierarchical cluster analysis (HCA)

In other to compare the five experimental groups, the volatile compounds (VCs) were subjected to HCA by clustering heatmap based on Euclidean distance and the Ward linkage method (Fig. 2). Columns and rows indicate each VC and different experimental groups, respectively. The darker red and darker green represent high and low contents, respectively. Shorter Euclidean distance indicates similarity of the samples. The results showed that the VCs were divided into four clusters. The first cluster consisted of terpenes (α-pinene, myrcene, γ-terpinene, α-terpinene, β-phellandrene, m-cymene, limonene, and phellandrene), aldehydes (hexanal, butanal, nonanal, and benzaldehyde), alcohols (1-hexanol, 2-methyl-1-butanol, 3-methyl-1-butanol, terpinene-4-ol), ester (methyl heptanoate), ketone (2-propanone), aliphatic (heptane, propylclopropane decamethyl-cyclopentasiloxane) and aromatic hydrocarbons (toluene). Overall, cluster covered most of VCs in all samples, being significantly higher in the chickpea nuggets formulated without pequi flour. However, some VCs including α-pinene,

methyl heptanoate, heptane, myrcene, butanal, 2-methyl-1-butanol, 3-methyl-1-butanol, nonanal, propylclopropane and toluene did not appear in the raw samples.

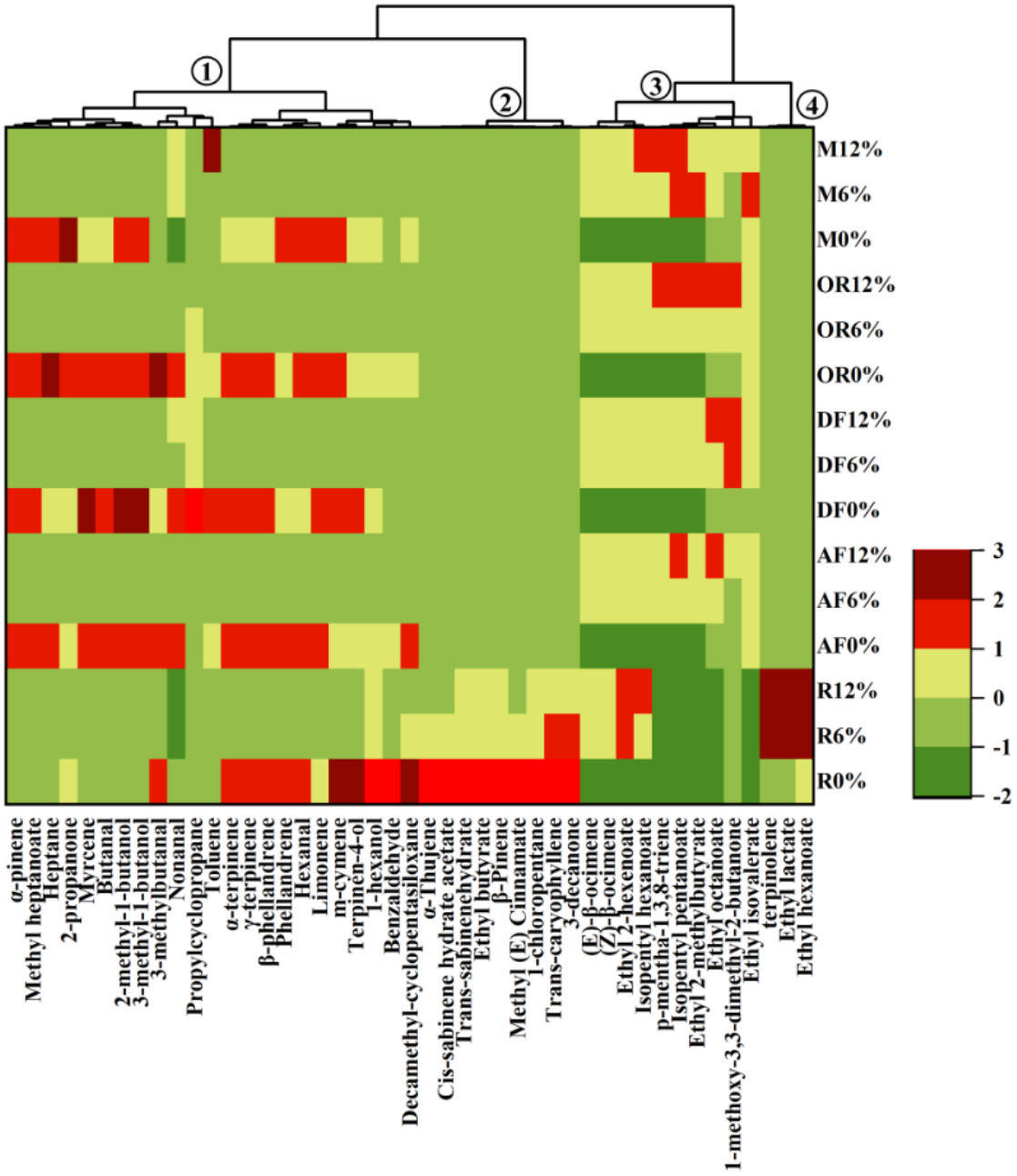


Fig. 2. Heat map of different volatile compounds of chicken nuggets treated with different levels of pequi flour and different cooking methods.

The second cluster consisted of α-thujene, cis-sabinene hydrate acetate, ethyl butyrate, β-pinene, methyl (E) cinnamate, 1-chloropentane, trans-caryoophyllene, and 3-decanone, α-Thujene, cis-sabinene hydrate acetate, ethyl butyrate, β-pinene, methyl (E) cinnamate, 1-chloropentane, trans-caryoophyllene, and 3-decanone.

These VCs were significantly higher in the raw samples than in the cooking samples, however, with the increase of PF in the raw samples, their contents decreased significantly ($p < 0.05$), except trans-caryoophyllene wich only decreased in the samples treated with 12% of PF. In the cooked chickpea nuggets, these VCs were not detected did. This may be because of the decomposition and volatilization of thermo-sensitive compounds at higher heating temperature (Boateng & Yang, 2021).

The third cluster was composed by (E)-β-ocimene, (Z)-β-ocimene, ethyl 2-hexanoate, isopentyl hexanoate, p-mentha-1,3,8-triene, isopentyl pentanoate, ethyl 2-methylbutyrate, ethyl octanoate, and 1-methoxy-3,3-dimethyl-2-butanoate and ethyl isovalerate. Most of these compounds were not detected in the control samples (without pequi) and were significantly higher in the cooked samples, being significantly accumulated in oven-roasted and microwaved samples than in the other cooking methods. The last cluster consisted of terpinolene, ethyl lactate and ethyl hexanoate. These VCs increased in the raw samples with increasing the levels of PF, however, in the cooked samples were not detected.

3.3.2. Principal component analysis (PCA)

PCA was carried out to highlight the differences between the VCs of the five experimental groups. The first two PC1 and PC2 with an eigenvalue of 22.26% and 13.12, respectively, accounted for 78.61% (49.46% and 29.15%, respectively) of total variance. PC1 was correlated with most VCs (Fig. 3A). According to the loading values (Table 5), (E)-β-ocimene, (Z)-β-ocimene, isopentyl hexanoate, ethyl 2-hexenoate, p-mentha-1,3,8-triene, isopentyl pentanoate, ethyl 2-methylbutyrate, and ethyl octanoate were negatively correlated with PC1, whereas m-cymene, phellandrene, γ-terpinene, β-phellandrene, α-terpinene, hexanal, terpinen-4-ol, limonene, decamethyl-cyclopentasiloxane, 2-methyl-1-butanol, 3-methylbutanal, and 1-hexanol were positively correlated with this component. On the other hand, PC2 was negatively correlated with methyl heptanoate, 3-methyl-1-butanol, butanal, α-pinene, heptane, ethyl isovalerate and myrcene.

On the other side, PC2 was positively associated with 3-decanone, trans-caryophyllene, β-Pinene, 1-chloropentane, methyl (E) cinnamate, α-thujene, trans-sabinenehydrate, cis-sabinene hydrate acetate, ethyl butyrate, 1-hexanol, and benzaldehyde. The results of the scores plot (Fig.) showed that the five treatments were divided into four groups with different VCs. Group 1 is located in the upper right side of the score plots was formed by raw chickpea nuggets

formulated without PF (R0%). This group was characterized by the following VCs: C7(phellandrene), C8(m-cymene), C13(β-pinene), C12(α-Thujene), C15(trans-caryophyllene), C24(ethyl butyrate), C26(*cis*-sabinene hydrate acetate), C27(*trans*-sabinenehydrate), C28(methyl(E) cinnamate), C31(1-hexanol), C32(terpinen-4-ol), C33(benzaldehyde), C38(3-decanone), C41(decamethyl-cyclopentasiloxane), C44(1-chloropentane). Group 2, is situated on the bottom right of the scores and consisted of different cooked samples formulated without PF (AF0%, DF0%, OR0%, and M0%).

This group was characterized by C1(α-pinene), C2(α-terpinene), C3(β-phellandrene), C4(γ-terpinene), C9(limonene), C10(myrcene), C20(methyl heptanoate), C29(2-methyl-1-butanol), C30(3-methyl-1-butanol), C34(butanal), C35(3-methylbutanal), C36(hexanal), C37(nonanal), C40(2-propanone), C42(propylcyclopropane), C43(heptane), C45(toluene). Group 3 is located in the left bottom of the scores plot and is formed by cooked samples formulated with 6% and 12% of PF (AF6%, AF12%, DF6%, DF12%, OR6%, OR12%, M6%, and M12%). This group is characterized by the following VCs: C11(p-mentha-1,3,8-triene), C17 (ethyl 2-methylbutyrate), C18 (ethyl isovalerate) C19 (ethyl octanoate), C22 (isopentyl pentanoate), and C39 (1-methoxy-3,3-dimethyl-2-butanone). Group 4 is placed in the upper left side of the scores plot and is composed of raw chickpea nuggets formulated with 6% and 12% of PF. This was correlated with C5((E)-β-ocimene), C6((Z)-β-ocimene), C14(terpinolene), C16(ethyl 2-hexenoate), C21(isopentyl hexanoate), and C23 (ethyl lactate), C25 (ethyl hexanoate).

Table 5 PCA loadings

Principal component	Eigenvalue	Total variance (%)	Cumulative variance (%)	Volatile compound	Loadings
PC1	22.26	49.46	49.46	(E)-β-ocimene	-0.98
				(Z)-β-ocimene	-0.97
				m-cymene	0.97
				Phellandrene	0.97
				γ-terpinene	0.97
				β-phellandrene	0.96
				α-terpinene	0.94
				Hexanal	0.94
				Terpinen-4-ol	0.92
				Isopentyl hexanoate	-0.91
				Limonene	0.90
				Ethyl 2-hexenoate	-0.84
				Decamethyl-cyclopentasiloxane	0.83

				p-mentha-1,3,8-triene	-0.82
				Isopentyl pentanoate	-0.82
				Ethyl 2-methylbutyrate	-0.81
				2-methyl-1-butanol	0.80
				3-Methylbutanal	0.76
				1-Hexanol	0.75
				Ethyl octanoate	-0.72
PC2	13.12	29.15	78.61	3-Decanone	0.90
				Trans-caryophyllene	0.88
				β-Pinene	0.84
				1-Chloropentane	0.84
				Methyl (E) Cinnamate	0.83
				α-Thujene	0.82
				Trans-sabinenehydrate	0.82
				Cis-sabinene hydrate acetate	0.82
				Ethyl butyrate	0.81
				Methyl heptanoate	-0.70
				3-methyl-1-butanol	-0.69
				Butanal	-0.69
				α-pinene	-0.68
				Heptane	-0.66
				Ethyl isovalerate	-0.66
				1-Hexanol	0.65
				Myrcene	-0.65
				Benzaldehyde	0.64

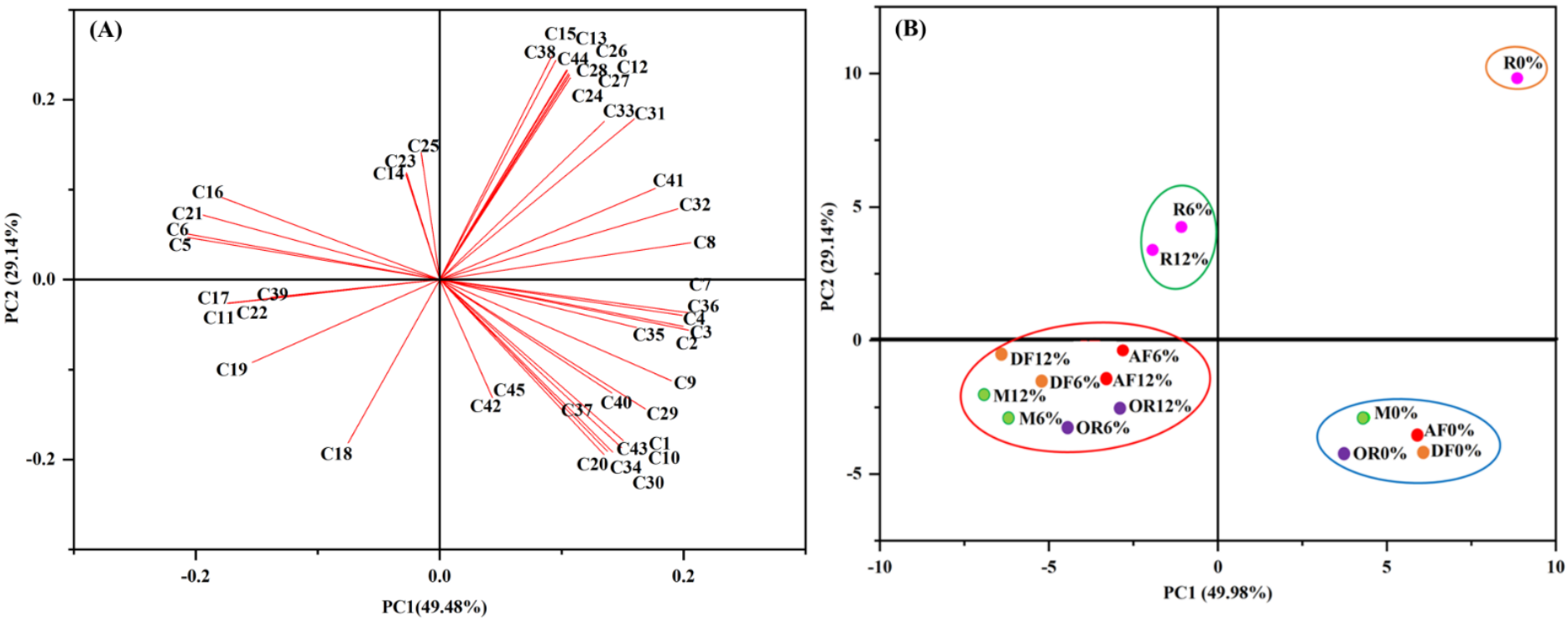
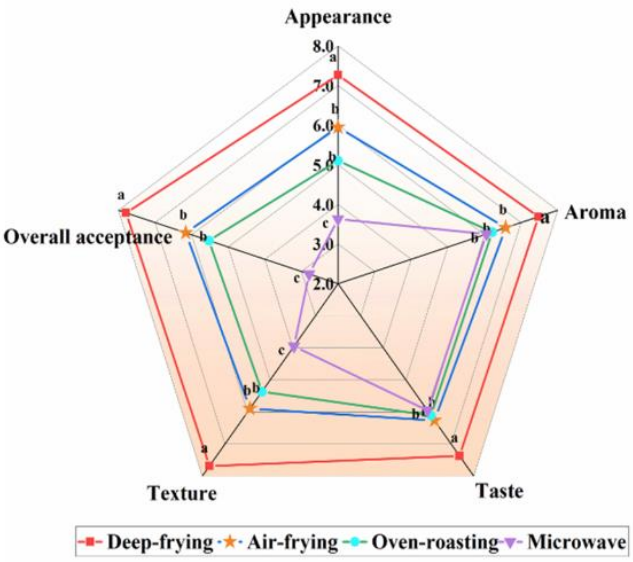


Fig. 3. Principal component analysis (PCA) of the volatile compounds of chicken nuggets. (A) Scores bi-plot (B) Loadings bi-plot. R: raw chicken nuggets; AF: air-fried chicken nuggets; DF: deep fried chicken nuggets; OV: oven-roasted chicken nuggets; M: microwaved chicken nuggets; 0%, 6% and 12% of PF; C1, α-pinene; C2, α-terpinene; C3, β-phellandrene; C4, γ-terpinene; C5, (E)-β-ocimene; C6, (Z)-β-ocimene; C7, phellandrene;C8, m-cymene; C9, limonene; C10, myrcene; C11, p-mentha-1,3,8-triene; C12, α-thujene; C13, β-pinene; C14, terpinolene; C15, trans-caryophyllene; C16, ethyl 2-hexenoate; C17, ethyl-2-methylbutyrate; C18, ethyl isovalerate; C19, Ethyl octanoate; C20, methyl heptanoate; C21, isopentyl hexanoate; C22, isopentyl pentanoate; C23, ethyl lactate; C24, ethyl butyrate; C25, ethyl hexanoate; C26, cis-sabinene hydrate acetate; C27, trans-sabinenehydrate; C28, methyl (E) cinnamate; C29, 2-methyl-1-butanol; C30, 3-methyl-1-butanol; C31, 1-hexanol; C32, terpinen-4-ol; C33, benzaldehyde; C34, butanal; C35, 3-methylbutanal; C36, hexanal; C37, nonanal; C38, 3-decanone; C39, 1-methoxy-3,3-dimethyl-2-butanone; C40, 2-propanone; C41, 2-propanone; C42, propylcyclopropane; C43, heptane; C44, 1-chloropentane; C45, toluene.

3.4. Sensory analysis

As shown in Fig. 4, deep-fried chicken nuggets received the highest scores for appearance, aroma, taste, texture and overall acceptance ($p < 0.05$), whereas no significant differences were observed between air-fried and oven-roasting samples ($p > 0.05$) for all sensory properties. On the other hand, microwaved samples had the lowest scores for appearance, texture and overall acceptance ($p < 0.05$), and no significant differences for aroma and taste were present among microwaved, air-frying and oven-roasting samples.



The higher appearance score of deep-fried chicken nuggets compared to the other cooking methods could be attributed to the uniform golden color generated on the surface of the chicken nuggets as a result of Maillard reaction and caramelization of carbohydrates from the ingredients used in coating process during deep-frying. Besides, the usage of oil during the deep-frying process increases the fat level of food products resulting in more attractive flavor and texture attributes that enhance the mouthfeel sensation of the consumers (Kupirovič et al., 2017).

These results suggests that deep-frying was the most acceptable cooking method, therefore, this method was selected for the next step of sensory evaluation. Similar to our results, (Tamsir et al., 2021) in their study with Malaysian fish sausage reported that deep-frying was the most preferred cooking method in terms of overall acceptability compared to air-frying, boiling, microwaving, oven-cooking and steaming.

4. Conclusions

The results of this study showed that incorporation of PF improved the cooking yield, carotenoids and monounsaturated fatty acids. However, the contents of polyunsaturated fatty acids were found to decrease. A total of 45 volatile compounds were detected in raw and cooked chicken nuggets, being terpenes and esters the most abundant volatile compounds. Air-frying resulted in the highest total carotenoids contents. Deep-frying is the most recommended cooking method since it was more preferred by consumers, along with a reasonable fatty acids and volatile profiles.

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ARTICLE 4: Effect of pequi (*Caryocar brasiliense* Camb.) flour incorporation on the physicochemical and sensory properties of plant-based meat analogue nuggets formulated with chickpea

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Abstract

This study aimed to evaluate the influence of pequi flour (PF) (0%, 3%, 6%, 9% and 12%) and storage time (0, 15, 30 and 45 days) on the quality of chickpea meat analogue (CMA) nuggets. Proximate composition, pick-up, oil absorption, water activity, pH, color, texture, lipid oxidation, and sensory properties by CATA test were investigated. In general, PF and time impacted the quality properties ($p < 0.05$). As PF increased, the values of lipids, total dietary fiber, ash, non-fiber carbohydrate, energy value, redness (a*), yellowness (b*), hardness, springiness, cohesiveness, chewiness, and TBARS increased, while the values of moisture, protein, pH, and lightness (L*) decreased; no significant differences were observed for pick up, aw, and oil absorption. Overall, increasing storage time led to decrease TBARS values, while the color and texture parameters fluctuated with an increase and decrease. The results of CATA test showed that the incorporation of PF had significant effect on the sensory attributes. Incorporation of PF up to 9% could be an effective strategy to develop functional CMA nugget.

Keywords: Functional food, *Cicer arietinum* L, quality, dietary source, carotenoids

1. Introduction

Meat and meat products have been essential components for human diet since ancient time, because they are major sources of high-quality protein with high digestibility and good essential amino acid profile; they are also a source of vitamins and minerals such as zinc and iron (Bis-Souza et al., 2019). According to the World Health Organization (WHO), the global population is predicted to reach 9.8 billion people by 2050, and the meat demand is expected to approach 45 billion tons (Kim et al., 2016). However, the growth rate of meat production is substantially lower than the population growth rate.

The meat production has been associated with several negative environmental impacts, such as greenhouse gas emission, pollution, degradation of the land, and freshwater (Köhler et al., 2019). Furthermore, in recent years, the number of vegans and vegetarians has been gradually increasing worldwide, owing to health, religious, ethical, and other reasons (Lee et al., 2022). In this sense, active research is being focused on the development of future foods that can replace conventional meat, and plant-based meat analogues hold promise as a possible approach.

Meat analogues, also known as meat substitutes, fake meat, alternative meat, faux meat, mock meat, and artificial meat, are more environmentally sustainable and are considered healthier due to their lower saturated fat content and cholesterol-free than real meat (Ismail et al., 2020). Plant proteins are the main ingredients used to formulate meat analogues, and soy protein is the most widely used one (Pietsch et al., 2019). However, the utilization of chickpea (*Cicer arietinum* L.) as meat analogue is unexplored.

Chickpea is one of the most important pulse crops grown and widely consumed around the globe, mostly know as a rich source of proteins with wide range of application in the food sector, owing to their low cost, high digestibility and well-balanced amino acid. The chickpea proteins also have other good functional properties, including solubility, emulsifying, gelling and foaming properties (Alu'datt et al., 2017; Sofi et al., 2020).

There are few studies about the use of chickpea to formulate meat analogues (Sharima-Abdullah et al., 2018). Previous studies reported that numerous bakery and meat products have been enriched by fiber rich powders to improve their nutritional value and functionality (Heo et al., 2019; Santos et al., 2022; Yadav et al., 2020). Limited studies are available about the incorporation of fiber-rich flours into plant-based meat analogues and their effect on the physico-chemical properties and the quality of the final product. In this regard, underexploited

native fruits from Brazil like pequi (*Caryocar brasiliense*), can be added to meat analogues during manufacturing process, resulting in high nutritional value and health benefits.

Pequi (*Caryocar brasiliense*) is a Brazilian fruit with huge amount of β-carotene. In recent years, this fruit has attracted more attention among consumers due to its unique aroma as well as its high nutritional value (Alves et al., 2010). Pequi is a rich source of dietary fiber, carbohydrates, and minerals such as Mg, Ca, Mn, and Cu (Silva et al., 2022). It is also an excellent source of phenolic compounds related to high antioxidant activity (Leão et al., 2017; Machado et al., 2015). More recently, studies have been associated the bioactive present in pequi with various functional properties like analgesic, ani-inflammatory, ameliorating aging-related and anticholinesterase (Braga et al., 2022; Junior et al., 2020; Roll et al., 2018). Thus, combining pequi flour and chickpea to develop meat analogues can be a good alternative to conventional meat-based food. However, to date, there have been no studies on developing nuggets analogues using chickpea incorporated with pequi flour.

Therefore, the objective of this research was to investigate the effect of pequi flour (PF) incorporation on the proximate composition, color, texture profile, lipid oxidation and sensory properties of plant-based meat analogue nuggets formulated with chickpea.

2. Material and methods

2.1. Materials

Frozen pequi fruit (*Caryocar brasiliense*) without the exocarp (a greenish-brown outer skin) and the white external mesocarp, harvested in 2021, were obtained from two Fruit Pulp Industries-Barra do Garças (Mato Grosso State, Brazil) and Goiânia (Goias State, Brazil). The fruits were then transported to the Pilot Plant in the Laboratory of Post-Harvest of Fruit and Vegetables Fruit of Federal University of Lavras (UFLA, Lavras, Brazil), where the experiment was carried out. All the ingredients, including the chickpea grains were obtained from local markets in Lavras, Brazil.

2.2. Preparation of pequi flour (PF)

Fruits consisted of the internal mesocarp and endocarp (yellowish-orange pulp) and seeds, without any physical injury and uniform in size and color were visually selected, sanitized with sodium hypochlorite solution (100 mg/L) for 15 min and then rinsed in distilled water to remove the residual chlorine. Afterward, they were bleached in a water bath at 80 ^{o}C

for 8 min. After 10 min cooling over in an ice bath, the fruits were then manually pulped using the stainless knife and the pulp and seeds were removed. Subsequently, the pulp was dried in an oven with forced air circulation (Solab SL 102 model, Piracicaba, São Paulo, Brazil) at 40ºC for 30 h. The dried pulp was first crushed using an industrial blender (Poli model LS-06, Brusque/Brazil), and then in a knife mill (Tecnal T650 model, Piracicaba, Brazil) and passed through a 20 – mesh sieve (0.85mm) to obtain the PF. The resulted flour was vacuum-packed in polyethylene plastic bags containing approximately 500g and stored frozen at -20 ºC until further use.

2.3. Formulation of chickpea meat analogue (CMA) nuggets

Five different formulations (Table 1) in three separated batches were prepared in the pilot plant of Laboratory of Meat Science and Technology at Federal University of Lavras. In brief, chickpeas were boiled for 20 min in a pressure cooker after overnight soaking (12h) at room temperature and ground in an electric grinder (BECCARO model PB-22, Rio Claro, Brazil), with 8 mm disc to obtain a pasta. The chickpea pasta was then mixed with all the other ingredients and additives. The final mixture was molded in a hamburger mold (11cm diameter), immediately frozen at -18 ºC for 8h, cut into four equal pieces (2.5 x 2.5 x 1.0 cm) and refrozen for 1h. After this, the formulated CMA nuggets were predusted with wheat flour, batter-coated with a mixture comprised of wheat flour, corn starch, salt and ice water, and breaded with dried bread crumbs. After coating, the CMA nuggets were pre-fried in soybean oil at 90 ºC for 30 s, immediately removed from the fryer and drained on absorbent papers at room temperature for 10min to remove the excess surface oil. Finally, the CMA nuggets samples were stored in polyethylene plastic bags and frozen at -18 ºC for 45 days. The physicochemical analyses were carried out at 0, 15, 30 and 45 days of frozen time and the sensory analysis was carried out only at first day of storage period.

Table 1 Formulations of the chickpea meat analogue (CMA) nuggets

Ingredients (%)	Treatments				
	PF0	PF3	PF6	PF9	PF12
Chickpea	95.0	92.0	89.0	86.0	83.0
Pequi flour	0.0	3.0	6.0	9.0	12.0
Salt (NaCl)	1.0	1.0	1.0	1.0	1.0
Phosphate	0.5	0.5	0.5	0.5	0.5
Textured soy protein	1.4	1.4	1.4	1.4	1.4
Sodium erythorbate	0.3	0.3	0.3	0.3	0.3
Garlic powder	0.3	0.3	0.3	0.3	0.3
Onion powder	0.3	0.3	0.3	0.3	0.3
White pepper powder	0.1	0.1	0.1	0.1	0.1
Nutmeg powder	0.1	0.1	0.1	0.1	0.1

PF0, CMA nuggets without pequi flour (control); PF3, PF6, PF9 and PF12, CMA nuggets with 3, 6, 9 and 12% pequi flour, respectively.

2.4. Heat treatment of chickpea meat analogue (CMA) nuggets

Frozen pre-fried CMA nuggets samples were thawed at room temperature and deep-oil fried using 2.5 L soybean oil in a pre-heated domestic deep-oil fryer at 180 ^{o}C for 4 min; the heat temperature and cooking time were chosen based on the preliminary experiment. After cooking, all samples were cooled down to the room temperature and used for the analyses.

2.5. Experimental design

A completely randomized design (CRD) with five levels of PF (0%, 3%, 6%, 9%, 12%) and three replicates was performed for analysis of proximate composition, oil absorption, pick-up, water activity and pH in raw CMA nuggets. Data from objective color, texture profile analysis (TPA), and TBARS were determined using a 5 x 4 factorial design, with five levels of pequi flour (0%, 3%, 6%, 9%, 12%) and four levels of storage time (0, 15, 30, 45 days). Sensory analysis data were evaluated using a completely randomized block design, including assessors as a random effect (repetitions).

2.6. Analyses

2.6.1 Proximate composition

The proximate composition analysis was carried in triplicate on CMA nuggets. The moisture, protein, ash and total dietary fiber were performed according to previous published methodologies (AOAC, 2019). For total moisture content (AOAC 950.46B), 10 g of the sample were dried to constant weight at 105 ^{o}C. Protein content (AOAC 981.10) was assessed by the Kjeldahl method and the factor 6.25 was applied for conversion of nitrogen to crude protein. The ashes were evaluated by combustion at a temperature of 550 ^{o}C (AOAC 920.153). The fat content determined by the cold extraction (chloroform/methanol/water) according to Bligh & Dyer (1959). The total dietary fiber content was analyzed by the enzymatic-gravimetric method (AOAC 991.43) using a Total Dietary Fiber Assay Kit. The non-fiber carbohydrate content was determined by subtracting the sum of moisture, protein, fat, ash, and total dietary fiber from 100%. Energy was calculated using the Atwater conversion factors (4 for carbohydrate and protein and 9 kcal for fat). The results were express in fresh matter.

2.6.2. Percentage of mass gain in the coating (pick-up) oil absorption

The pick-up was obtained by the difference between the mass of nuggets (10 samples per treatment) before and after the three-step coating process (pre-dust, batter, and breading) following the equation (1). Oil absorption was determined by the difference between the mass of the pre-fried and raw samples according to equation (2).

$$\text{Pick-up (\%)} = \left(\frac{\text{Nuggets with coating - Nuggets without coating}}{\text{Nuggets with coating}} \right) \text{x } 100 \qquad (1)$$

$$\text{Oil absorption (\%)} = \left(\frac{\text{Weight pre-fried nuggets - Weight raw nuggets}}{\text{Weight pre-fried nuggets}} \right) \text{x } 100 \qquad (2)$$

(3)

2.6.3. pH and water activity

The pH and water activity (a_w) were performed in triplicates, in raw samples, at zero storage period. 5g of nugget samples were homogenized with 45 mL of distilled water for 2 min and the pH was determined using a pH meter (Tecnal TEC-3MP). Water activity of the nuggets was measuring using an Aqualab Water Activity Meter (Decagon Devices, Inc., Pullman, WA, USA) at 25 ^{o}C.

2.6.4. Texture profile analysis (TPA) and instrumental color

The instrumental texture profile analysis (TPA) and instrumental color were performed on the cooked CMA nuggets. Prior to analysis, the samples were cooled down to room temperature for 30 min, cut into cubes of 2x2 cm edge and the coating system was removed to avoid the potential interference on the results of TPA parameters and to evaluate the effect of the PF on the internal color of the CMA nuggets. TPA parameters (hardness, springiness, cohesiveness and chewiness) were investigated using a Texture Analyzer (TA-XT2i Stable Micro Systems, Godalming, UK) equipped with a 6 mm diameter cylindrical aluminum probe, at a constant speed of 2 mm/s and the samples were compressed twice to 50% of their original height with a compression load cell of 5kg.

The effect of the PF on the internal color of the formulations were assessed using a Minolta CR-400 (Konica Minolta Sensing, Inc., Osaka, Japan) colorimeter, operating with D65 illuminant, 8 mm aperture, and 10^{o} observer angle. The color indexes of the CIElab system L* or lightness (L* 0 = black; 100 = white), a* (redness) and b* (yellowness) were recorded at 10 points for each treatment.

2.6.5. Lipid oxidation

Lipid oxidation of samples during storage period was performed using the thiobarbituric acid reactive substances (TBARS) as described by Jo & Ahn (1998). TBARS values were determined using the standard curve generated by using 1,1,3,3, tetra ethoxypropane standard calibration curve and reported as mg malonaldehyde (MDA)/kg sample.

2.6.6. Sensory analysis

To assess the sensory attribute of the CMA nuggets, CATA along with hedonic acceptance tests were conducted as described by Guimarães et al. (2022), with small changes. The analyses were carried out at the sensory analysis laboratory of the Federal University of Lavras (UFLA). Prior to that, the research was approved by the institutional ethics commission (code of ethics: CAAE: 55582822.6.0000.5148) and the informed consent was obtained from the panelists. For selecting the CATA attributes, a preliminary test with a focus group consisted of 17 untrained nuggets consumers of both genders (10 non-vegetarians and 7 vegetarians) was performed. Each panelist individually tasted samples of CMA nuggets and in the group, they discussed the attributes until a consensus was reach. The final attribute list comprised 32 terms

(Table 2) grouped into 5 categories, including appearance (10), aroma (7), flavor (6), taste (3), and texture (3).

Then, a total of 60 untrained assessors, among undergraduate and post-graduate students, staff and professors, aged between 18 and 60 years (68% female and 32% male) were randomly recruited at UFLA on the basis of their interest, availability and habit in consuming nuggets or vegetarian food, at least once a month. Most of the participant (90%) in this study declared that they were not vegetarian or vegan. A total of 45% assessors reported the consumption of nuggets once a week, twice a week, or twice a month, while a total of 42% assessors reported consumption of vegetarian or vegan products once a week, twice a week, or twice a month.

The tests were carried out in individual booths under fluorescent lighting. Samples were cut into cubes of 2 cm edge, coded with random three digits numbers and served to consumers in balanced order, following a monadic way. Room temperature water was used to clean the palate between each evaluation. Before the analysis, the judges were instructed to read the attributes and, after they tried the samples, they were asked to select without limit all the attributes they considered appropriate to describe each sample. Along with CATA test, consumers were asked to perform the acceptance test in terms of appearance, aroma, flavor, texture, and overall acceptance using a structured 9-point hedonic scale (1 = “dislike very much” to 9 = “like very much”). Besides, a 5-point hedonic scale (1 = certainly would not buy; 5 = certainly would buy) was also used to evaluate the purchase intention of CMA nuggets

2.7. Statistical analysis

Data were reported as mean ± standard deviation. Analysis of variance (ANOVA) was carried out using R version 4.0.4 (R Core Team, 2020). Significant differences between means were investigated by Tukey’s average test ($p < 0.05$). Correspondence Analysis (CA) and Cochran’s Q test were used to evaluate the descriptors terms of CATA.

3. Results and discussion

3.1. Proximate composition, pH, oil absorption, pick-up and aw

All the proximate composition variables, energy and pH values of the chickpea meat analogue (CMA) nuggets had changed significantly ($p < 0.05$) with the addition of pequi flour (PF), except for pick-up, water activity and oil absorption values, where no significant differences ($p > 0.05$) from the control samples were noted. The moisture content of CMA nuggets showed a significant decrease from 62.64 to 56.8 g/100g with the incorporation of PF. This reduction can be attributed to the stronger water retention and the low moisture content (6.72g/100g) of PF.

The lipids contents increased (6.13-10.98 g/100g) with the addition of PF due to lipid rich PF with a lipid content around 66.40 g/100g as compared to chickpea flour. In our study, increasing levels of PF resulted in the significant decrease of protein content from 18.23 to 10.60 g/100g, which might be due to the lower protein content in PF in comparison with chickpea with protein content of 24 g/100g (Sofi et al., 2020).

The ash contents significantly increased in the enriched CMA nuggets as compared to control samples. This result suggested that PF is an excellent source of minerals. In this study, it was found that total dietary fiber (TDF) significantly increased (8.27-11.23 g/100g with increasing of PF. This increase might be due to PF high in TDF content (12.56 g/100g) (Silva et al., 2022).

In general, the non-fiber carbohydrate content and energy value of CMA nuggets showed significant ($p < 0.05$) increase (3.53-7.69 g/100g and 142.16-172.01 Kcal/100g, respectively) with the increase proportion of PF. The increase in the energy value was mainly due to the increase of lipid content. As the incorporation of PF increased, the pH values significantly decreased in range from 6.63 to 6.23, due to the lower pH of PF added.

Table 2 Effects of different levels of pequi flour (PF) on the proximate composition, emulsion stability, oil absorption, pick-up, a_w and pH of chickpea meat analogue (CMA) nuggets.

Analysis	Treatments				
	PF0	PF3	PF6	PF9	PF12
Proximate composition (g/100g)					
Moisture	62.64 ± 0.07^{a}	59.58 ± 0.12^{c}	60.93 ± 0.25^{b}	59.79 ± 0.15^{c}	56.87 ± 0.14^{d}
Lipids	6.13 ± 0.14^{c}	7.88 ± 0.23^{b}	8.60 ± 0.12^{b}	9.03 ± 0.20^{b}	10.98 ± 0.93^{a}
Protein	18.23 ± 0.28^{a}	16.27 ± 0.23^{b}	14.41 ± 0.73^{c}	12.55 ± 0.46^{d}	10.60 ± 0.62^{e}
Ash	1.21 ± 0.01^{c}	1.27 ± 0.11^{c}	1.58 ± 0.35^{bc}	2.13 ± 0.11^{ab}	2.62 ± 0.27^{a}
Total dietary fiber (TDF)	8.27 ± 0.39^{b}	8.95 ± 0.35^{b}	9.26 ± 0.20^{ab}	10.12 ± 1.28^{ab}	11.23 ± 1.26^{a}
Non-fiber carbohydrate	3.53 ± 0.46^{b}	6.05 ± 0.46^{ab}	5.23 ± 0.13^{ab}	6.37 ± 1.80^{a}	7.69 ± 1.03^{a}
Energy value (Kcal/100g)	142.16 ± 1.41^{c}	160.17 ± 1.41^{ab}	155.92 ± 3.12^{bc}	156.98 ± 4.71^{b}	172.01 ± 10.75^{a}
Oil absorption (%)	3.02 ± 0.61^{a}	2.77 ± 0.22^{a}	2.99 ± 0.37^{a}	2.84 ± 0.25^{a}	2.92 ± 0.37^{a}
Pick-up (%)	29.15 ± 1.86^{a}	30.24 ± 2.20^{a}	29.37 ± 2.91^{a}	31.06 ± 2.77^{a}	30.87 ± 3.13^{a}
Water activity (aw)	0.97 ± 0.01^{a}	0.96 ± 0.02^{a}	0.96 ± 0.01^{a}	0.96 ± 0.02^{a}	0.94 ± 0.01^{a}
pH	6.63 ± 0.01^{a}	6.52 ± 0.01^{b}	6.37 ± 0.01^{c}	6.36 ± 0.01^{c}	6.23 ± 0.01^{d}

Data are reported as means ± standard deviation (n = 3). PF: pequi flour; CMA: chickpea meat analogue; PF0: control CMA nuggets (without PF); PF3, PF6, PF9 and PF12: samples with 3, 6, 9 and 12% PF incorporation, respectively. Mean with different letters in a row are significantly different (*$p < 0.05$) by Tukey test. n.s – non-significant

3.2. Instrumental color parameters

Color is one of the crucial food quality attributes that can influence the perception and consumer preference of the product (Kamani et al., 2019). Fig. 1 presents the internal color measurements of the CIElab system (L*, a*, b*) for CMA nuggets. The enriched CMA nuggets resulted in a significant reduction in lightness (L*) and an increase in redness (a*) and yellowness (b*) with the increase incorporation of PF from 3 to 12% compared to control samples.

Changes in the instrumental color parameters of plant-based nuggets have also reported by other researchers. For example, Sharima-Abdullah et al. (2018) have also reported a decrease in L* value and an increase in a* value of imitation chicken nuggets formulated with different concentrations of chickpea flour and textured vegetable protein. However, the b* values decreased as the proportion of chickpea flour to textured vegetable protein reduced. Moreover, Husain & Huda-Faujan (2020) recently reported a decrease in L* value of imitation chicken nuggets with decreasing the percentage of grey oyster mushroom stems to chickpea flour, while no significant differences ($p > 0.05$) were found between treatments for a* and b* values. In our study, significant changes in the instrumental color parameters of CMA nuggets with the increase percentage of PF might be related to the orange-yellow color of added PF as well as the Maillard reaction products and caramelization process (Silva et al., 2022).

With regard to storage time, overall, it was noted that L* values of all treatments did not differ significantly from each other, although a slight increase in this parameter was observed between 15 and 30 days for CMA nuggets formulated with 9% of PF. On the other hand, no significant differences in a* values (Fig. 2B) were observed among all treatments during the storage time. However, the b* values (Fig. 2C) did not show significant changes up to 30-day storage and then decreased thereafter. This decrease might be related to the protein and lipid oxidation.

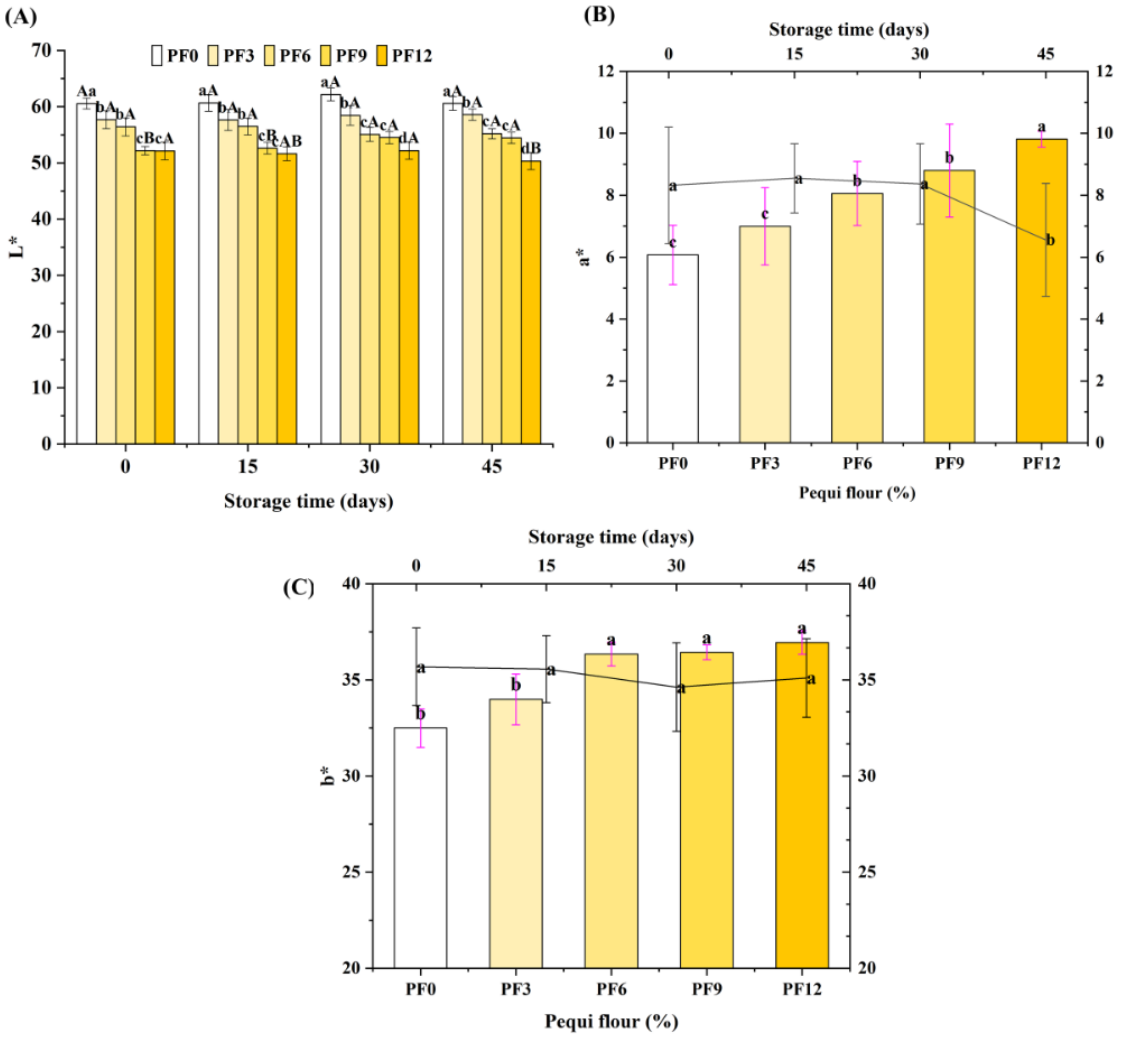

Fig.1. Effects of different levels of pequi flour and storage time on the objective color (L*, a* and b*) of chickpea meat analogue (CMA) nuggets during storage for 45 days. PF: pequi flour; PF0: CMA analogue with 0% of PF (control); PF3, PF6, PF9 and PF12: CMA nuggets with 3, 6, 9 and 12% of PF incorporation, respectively. Different letters (A-B) indicate significant differences between different levels of PF (bars with different colors) for the same storage time ($p < 0.05$), while different letters (a-c) indicate significant differences between different storage time for the same level of PF (bars with the same color) ($p < 0.05$) by Tukey test.

3.3. Texture profile analysis (TPA)

Routine instrumental tests most often used in meat products evaluation can also be applied to judge the sensory characterization of plant-based meat analogs. Among these tests, TPA is found to be suitable to detect texture differences of meat analogs products, including hardness, springiness, cohesiveness, and chewiness (Huang et al., 2022). As shown in Fig. 2, all the TPA attributes (hardness, springiness, cohesiveness, and chewiness) were impacted by both PF and storage time ($p < 0.05$). There was a significant interaction between PF and storage time ($p < 0.05$) only for cohesiveness.

In general, the hardness, springiness, cohesiveness, and chewiness of CMA nuggets significantly increased ($p < 0.05$) with increasing the percentage of PF. This increase can be attributed to the synergistic effect of the fiber content from chickpea and PF, which can act as a thickening and gelling agent, to form a stronger gel matrix with high water binding capacity, resulting in harder and chewier CMA nuggets (Fang et al., 2019; Xiong et al., 2022). Moreover, this finding might also be related to the water loss during cooking which led to an increase in protein concentration, thereby promoting more protein-protein interactions (Vu et al., 2022). Faujan et al. (2018) also reported the increase of hardness, chewiness, springiness, and cohesiveness of imitation chicken nuggets with increasing the levels of textured vegetable protein and decreasing of chickpea flour. During storage time, the hardness (Fig. 2A) decreased first up to 30 days followed by an increase thereafter.

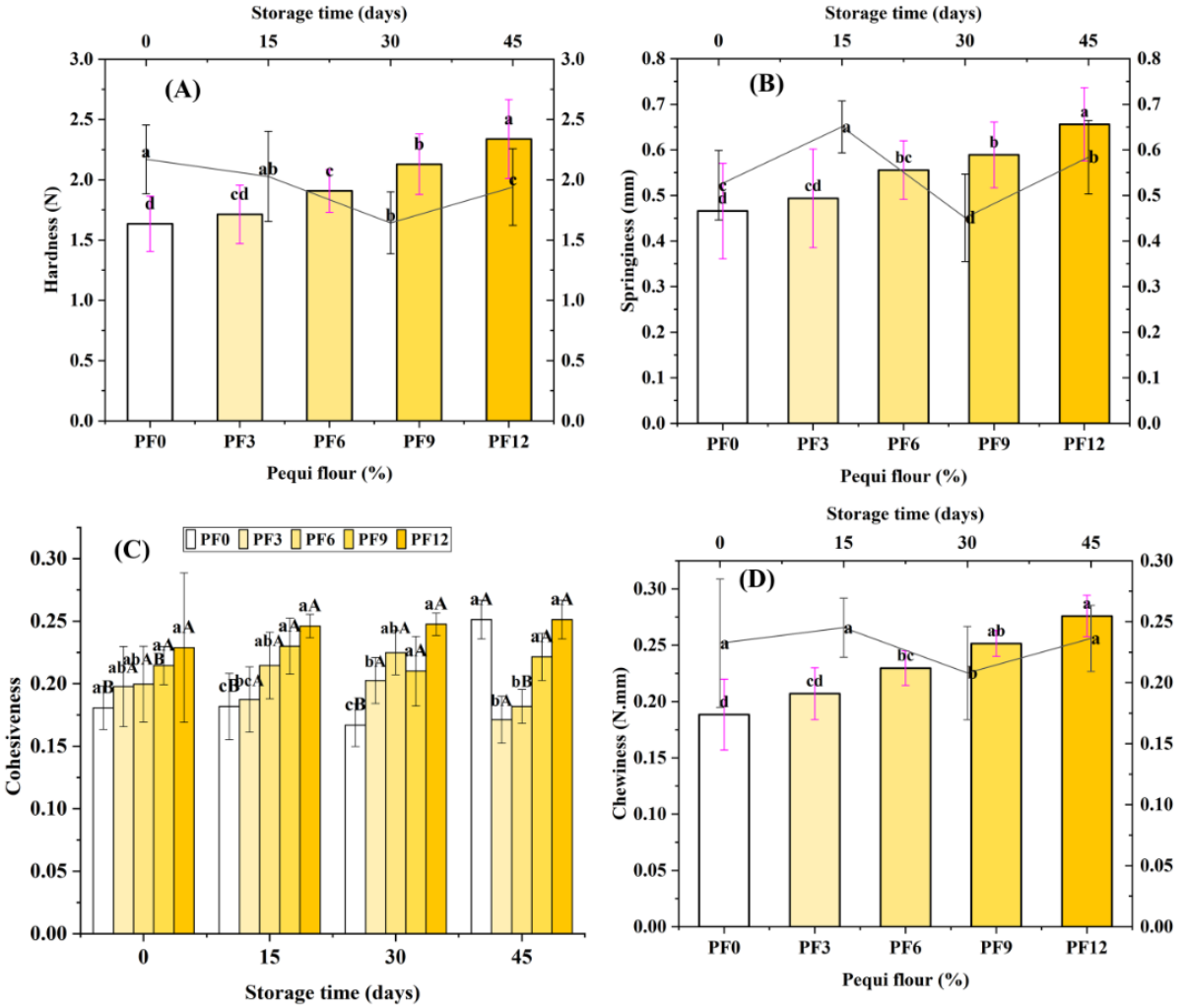

Fig.2. Effects of different levels of pequi flour and storage time for 45 days on the texture profile parameters of the chickpea meat analogue (CMA) nuggets during storage for 45 days. PF: pequi flour; PF0: CMA analogue with 0% of PF (control); PF3, PF6, PF9 and PF12: CMA

nuggets with 3, 6, 9 and 12% of PF incorporation, respectively. Different letters (A-B) indicate significant differences between different levels of PF (bars with different colors) for the same storage time ($p < 0.05$), while different letters (a-c) indicate significant differences between different storage time for the same level of PF (bars with the same color) ($p < 0.05$) by Tukey test.

On the other hand, the springiness and chewiness (Fig. 2C, 2D) fluctuated with storage period; increased first, then decreased, and then increased, however, no significant effect on the cohesiveness were found in enriched CMA nuggets over 45 days of storage period, except in control samples where the cohesiveness values dramatically increased, particularly after 30 days until the end (Fig. 2C). Theses outcomes suggested that the storage time significantly impacted the texture attributes of CMA nuggets. These changes in the texture attributes during storage could be caused by several factors such as water binding capacity, changes in pH, oxidation and modification of the protein structures as well as their interactions with other food ingredients that can affect their functionalities (Delmas & Barthe, 2015; Xu et al., 2020).

3.4. Lipid oxidation

Lipid oxidation is one of the most important indicator linked with the shelf life and quality of high-fats foods (Pateiro et al., 2018). The TBARS (2-thiobarbituric-acid-reactive substances) assay is widely performed to estimate the degree of lipid oxidation, particularly of high-fat foods, and it is used to indicate the levels of secondary products of lipid oxidation (Luo et al., 2022). The minimum acceptable limit of TBARS for a good quality food product is < 1.0 mg MDA/kg (Reitznerová et al., 2017). As shown in Fig. 3A and B, the TBARS values of both raw and cooked CMA nuggets were substantially ($p < 0.05$) affected by the concentration of PF and storage time and the interaction between these two factors.

In general, the TBARS values of all chickpea nuggets increased with increasing the concentration of PF ($p < 0.05$), indicating that the addition of PF promoted lipid oxidation in the CMA nuggets. However, the TBARS values of all CMA nuggets (raw and cooked) significantly ($p < 0.05$) decreased during the entire storage time. Although pequi is a rich source of bioactive compounds including phenolics, tocopherols, phytosterols and carotenoids, the presence of high amount of unsaturated fatty acids, especially oleic acid, which are highly susceptible to oxidation might have contributed to increase the TBARS values, thereby resulting in the lipid oxidation of CMA nuggets.

Moreover, since the phenolic compounds can initiate and auto-oxidation process, pequi flour could finally behave like pro-oxidant (Sirini et al., 2020). Similar behavior was also reported by Xiong et al. (2022) in beef sausages formulated with sorghum bran. On the other side, the decrease of TBARS values during storage time could be attributed to the antioxidant effect of the chickpea. However, the TBARS obtained in all samples were within the acceptable limit for consumer acceptability.

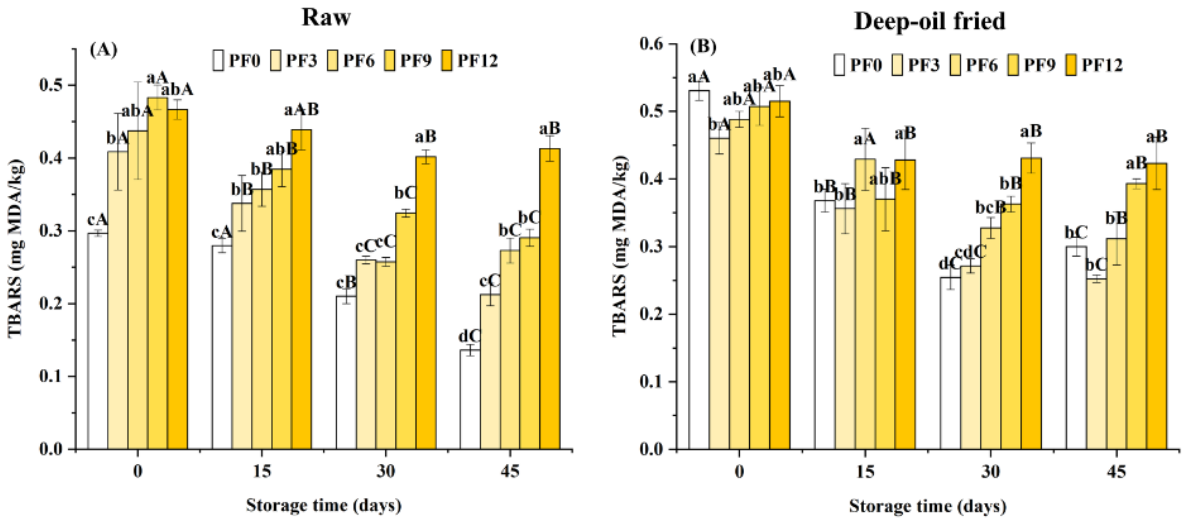

Fig.3. Effect of different levels of pequi flour and storage period (45 days) on the TBARS values of the raw and cooked chickpea meat analogue (CMA) nuggets. PF: pequi flour; PF0: CMA analogue with 0% of PF (control); PF3, PF6, PF9 and PF12: CMA nuggets with 3, 6, 9 and 12% of PF incorporation, respectively. Different letters (A-B) indicate significant differences between different levels of PF (bars with different colors) for the same storage time ($p < 0.05$), while different letters (a-c) indicate significant differences between different storage time for the same level of PF (bars with the same color) ($p < 0.05$) by Tukey test.

3.5. Sensory analysis

The effect of PF on the sensory attributes of CMA nuggets is presented in Fig. 3B and C. All the attributes were significantly ($p < 0.05$) affected by the incorporation of PF, except appearance. Overall, all the samples were well accepted with the exception of samples formulated with 12% of PF. Aroma, taste, texture, overall acceptance (Figand purchase intention scores increased with increasing PF up to 9% followed by decrease in the samples treated with 12% of PF. The results indicated that the addition of up to 9% PF in chickpea CMA nuggets can be recommended without compromising their acceptability by consumer, however, for the preference, samples formulated with 9% were the most chosen.

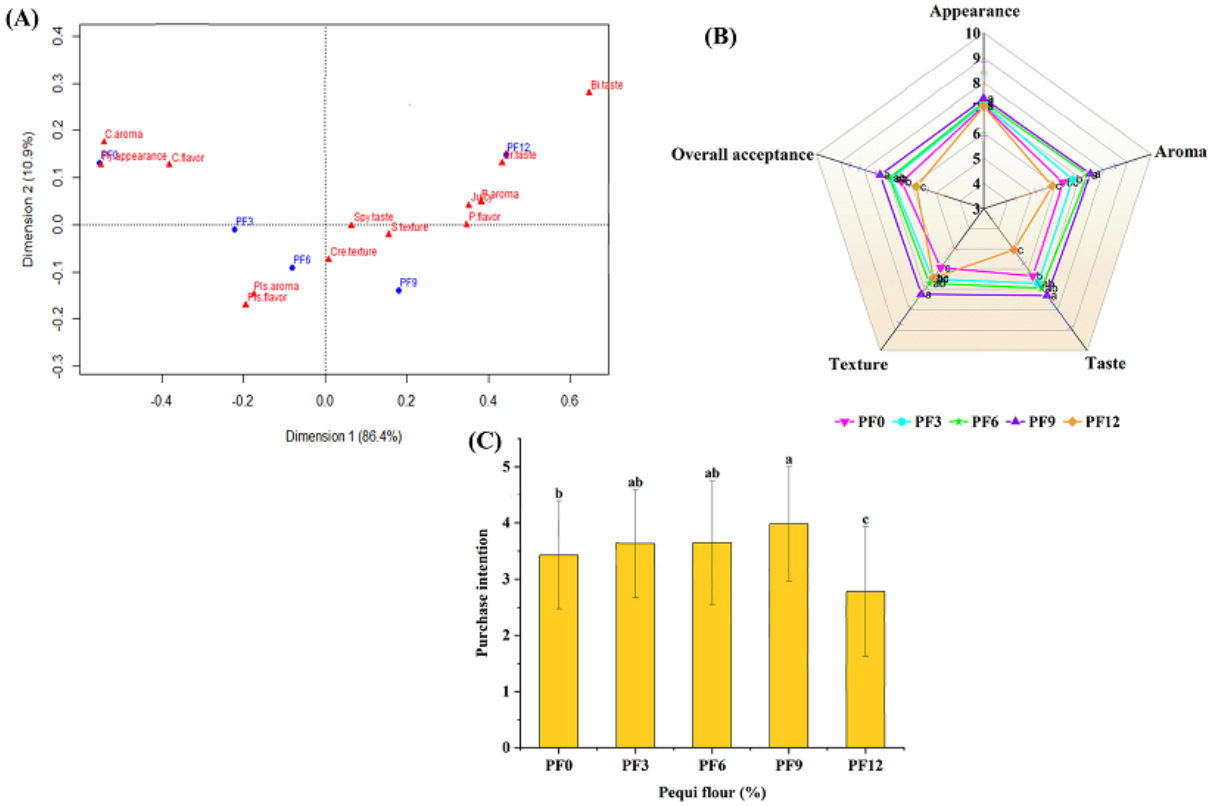

Fig.4. Correspondence analysis map of the CATA descriptors terms in the first two dimensions (A) and the effect of different levels of pequi flour (B and C) on the sensory attributes of chickpea meat analogue (CMA) nuggets. PF: pequi flour; PF0: CMA analogue with 0% of PF (control); PF3, PF6, PF9 and PF12: CMA nuggets with 3, 6, 9 and 12% of PF incorporation, respectively. Bi.taste: bitter taste; Br.taste: bitter residual taste; P.aroma: pequi aroma; P.flavor: pequi flavor; S.texture: soft texture; Spy.taste: spicy taste; Cre.texture: creamy texture; Pls.flavor: pleasant flavor; Pls.aroma: pleasant aroma; C.aroma: chickpea aroma; Py. appearance: pale yellowish appearance.

The results of CATA test showed that there were significant differences ($p < 0.05$) only for 13 of the 32 terms used to describe the samples, according to the nonparametric Cochran's Q test. These 13 terms included two of appearance (pale yellowish appearance, and yellowish color), three of aroma (pequi aroma, pleasant aroma and chickpea aroma), three of flavor (pequi flavor, chickpea flavor, and pleasant flavor), two of taste (bitter taste and bitter residual taste), and three of texture (creamy texture, soft texture, and juicy) terms. These results suggested that these terms had significant contribution to discriminating the different chickpea nuggets. In order to highlight the differences between the formulations the correspondence analysis (CA) was carried out with the 13 significant attributes. The first two dimensions of the CA explained 97.3% of the total variance of the experimental data (86.4% and 10.9%, respectively).

The treatments were separated into three groups with different sensory characteristics. The group located in the negative part of the first dimension was formed by the control samples (without PF) and samples formulated with 3% of PF. This group was correlated with the following terms: chickpea aroma, pale yellowish appearance and chickpea flavor. The chickpea samples formulated with 12% of PF were located in the positive quadrant of the first dimension and were characterized by pequi flavor, spicy taste, juicy, bitter taste, pequi aroma, bitter residual taste and soft texture.

Finally, the CMA formulated with 6% and 9% of PF were located in the negative part of the second dimension and were characterized by the following terms: creamy texture, pleasant aroma and pleasant flavor. These results indicated that the addition of PF had a strong impact on the sensory attributes of CMA nuggets.

Table 2 Frequency citations of the check-all-that-apply (CATA) descriptors terms of chickpea meat analogue (CMA) nuggets with different levels of pequi flour (PF)

Attributes	Terms	Treatments				
		PF0	PF3	PF6	PF9	PF12
Appearance	Homogeneous internal appearance (Hi.appearance)^{ns}	38	31	29	34	24
	Frying appearance (Fr.appearance)^{ns}	43	42	48	49	44
	Pale yellowish appearance (Py.appearance)*	16	13	12	6	5
	Golden color external appearance (G.appearance)^{ns}	26	30	28	37	27
	Crumbly appearance (Cru.appearance)^{ns}	5	5	6	8	14
	Nugget appearance (Nug.appearance)^{ns}	34	28	28	29	25
	Yellowish color (Y.color)***	15	26	34	36	42
	yellow dots (Y.dots)^{ns}	5	14	10	13	12
	Crunchy appearance (Chy.appearance)^{ns}	32	24	33	32	25
	Pale-whitish appearance (Pw. appearance)^{ns}	4	7	3	3	4
Aroma	Pequi aroma (P.aroma)***	8	16	17	33	40
	Frying aroma (Fry.aroma)^{ns}	40	36	35	40	40
	Bittersweet aroma (Bs.aroma)^{ns}	5	11	7	7	11
	Spicy flavor (Spy.flavor)^{ns}	1	2	4	3	6
	Pleasant aroma (Pls.aroma)**	28	35	37	42	21
	Chickpea aroma (C.aroma)***	35	30	20	14	12
	Nugget aroma (Nug.aroma)^{ns}	28	16	21	16	18
Flavor	Pequi flavor (P.flavor)***	8	23	24	39	46
	Chickpea flavor (C.flavor)*	31	28	24	17	17
	Spicy flavor (Spicy flavor)^{ns}	27	34	32	30	24
	Pleasant flavor (Pls.flavor)**	26	31	34	39	17

	Wheat flour flavor (Wf.flavor)^{ns}	13	18	12	13	11
	Corn flour flavor (Cf.flavor)^{ns}	8	10	11	11	11
Taste	Bitter taste (Bi.taste)***	1	3	4	7	15
	Spicy taste (Spy.taste)^{ns}	4	7	9	7	9
	Bitter residual taste (Br.taste)**	3	5	6	11	16
Texture	Crunchy crust texture (Chy.texture)^{ns}	47	43	41	43	35
	Moist looking texture (Ml.texture)^{ns}	47	43	43	42	43
	Creamy texture (Cre.texture)*	19	34	32	38	32
	Soft texture (S.texture)***	15	25	31	35	38
	Crumbly texture (Cru.texture)^{ns}	4	8	9	14	12
	Juicy***	7	13	19	26	34

Data are reported as means ± standard deviation (n = 3). PF: pequi flour; PF0: CMA analogue with 0% of PF (control); PF3, PF6, PF9 and PF12: CMA nuggets with 3, 6, 9 and 12% of PF incorporation, respectively. Significant difference for *$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$ (Cochran´s Q test)

^{ns} non-significant difference according ($p > 0.05$) according to Cochran's Q test.

4. Conclusions

In this study, results demonstrated that incorporation of PF up to 9% could be a viable alternative to develop CMA nuggets with good sensory properties without affecting the acceptance of the product. Inclusion of PF increased the contents of total dietary fiber, lipids, ash, non-fiber carbohydrate, energy, and values and all the texture parameters. The TBARS values increased with the increasing incorporation of PF and decreased during the storage time. Therefore, PF can be used to improve the functional properties of CMA nuggets.

Acknowledgments

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ARTICLE 5: Effect of pequi (*Caryocar brasiliense* Camb.) flour and cooking methods on the quality characteristics of chickpea meat analogue nuggets.

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Abstract

Several imitation products have been developed with the rapid expansion of meat analogues. This study investigated the effect of different concentrations of pequi flour (PF) and different cooking methods on the cooking loss, carotenoids, fatty acid profile, volatile compounds and sensory attributes of chickpea meat analogue (CMA) nuggets. Overall, the addition of PF increased the contents of carotenoids, monounsaturated fatty acids (MUFA), saturated fatty acids (SFA), terpenes, and esters, while decreased the cooking loss and the contents of polyunsaturated fatty acids (PUFA). Air-frying and microwave resulted in the highest retention of carotenoids. Deep-oil frying exhibited the highest PUFA, MUFA and the lowest SFA, aldehydes, atherogenic index (AI) and thrombogenic index (TI). Deep-oil fried CMA nuggets were more acceptable, thus being suggested as the best cooking method.

Keywords: Plant based; *Cicer arietinum* L; fatty profile, volatile compounds; carotenoids, sensory properties.

1. Introduction

Since ancient times, meat and meat products have played a pivotal role in human diet, due to their high nutritional value; they represent a major source of proteins with high biological value, vitamins and mineral such as iron, and zinc (Bis-Souza et al., 2019). Global meat and meat products consumption has been considerably increased over the last 50 years due to rapid population growth, urbanization, and rise in personal incomes (Seto & Ramankutty, 2016). It is predicted that the world population will reach 9.8 billion people by 2050, and the demand for animal-based food is expected to increase by 70% (Choudhury et al., 2020).

The increasing meat products consumption prompts livestock farming and the production of animal-based food, resulting in several impacts on the environment including deforestation, biodiversity loss, greenhouse gas emissions and other forms of pollution, limited natural resources, water use and eutrophication (Crippa et al., 2021; González et al., 2020). Moreover, in recent years consumers are more aware of the negative impacts of consuming high sodium levels, fat, and harmful additives related to processed meat consumption (Thangavelu et al., 2022).

All the above-mentioned concerns along with the ethical issues and the shift towards niches such as vegan and vegetarian are leading to increasing interest in alternative source of protein (Kazir & Livney, 2021; Padilha et al., 2022). In this regard, plant-based meat analogues have been highlighted as one sustainable approach and an excellent strategy to replace traditional animal-based foods (McClements & Grossmann, 2021). Plant-based alternative sector is growing exponentially and has become prevailing trend (Vaikma et al., 2021). In monetary terms, the global plant-based meat sales accounted for $12.1 billion in 2019 and is predicted to grow by 15% to reach $27.9 billion by 2025 and $140 billion by 2029 (Richter, 2019).

It has been reported that plant-based food with high level of dietary fiber, oligosaccharides, and polyunsaturated fatty acids are linked to a lower risk of obesity and cardiovascular disease (Gu et al., 2022). Furthermore, replacing red meat with plant-protein substitutes, especially legume protein, has been reported to reduce the risk of type II diabetes recent study (Gu et al., 2022). Plant-based meat analogues are commonly designed to mimic the sensory properties of the most popular meat products such as nuggets, sausages, and patties, so that consumers can easily incorporate them into their dietary patterns (He et al., 2020).

Plant proteins including soy protein, wheat gluten and pea protein are currently the most common ingredients used to production meat analogues, which are usually used in form of isolates and concentrates (He et al., 2020; Shaghaghian et al., 2022). However, the use of other protein sources like chickpea in the formulation of meat analogue is still scarce. Chickpea (*Cicer arietinum* L.) is a rich source of high-quality proteins for formulating meat analogues. Chickpea contains high levels of protein (20.6-26.7%), carbohydrate (54-66%), dietary fiber (12.0%) and low concentrations of fat (3.1-7.0%) (Chibbar et al., 2010).

Chickpea proteins have wide range of applications due to their low cost, high digestibility and a good balance essential amino acid profile, which make it a good candidate for meat analogue formulations (Boukid, 2021; Faridy et al., 2020). Moreover, chickpea proteins have good functional properties, including water holding, solubility, emulsifying, gelling and foaming capacities; in this sense, food formulated with chickpea result in better functional and different sensory properties (Shaabani et al., 2018; Sofi et al., 2020). Chickpea has also potential health benefits such as prevention of cardiovascular disease, hypo-cholesterolemic, anti-diabetic, anti-cancerous, and anti-inflammatory activity (Kaur & Prasad, 2021).

Pequi (*Caryocar brasiliense* Camb.), also known as pequi, is an unexplored native fruit from Brazilian Cerrado widely used in local cooking owing to its unique flavor and pleasant taste (Silva et al., 2020). Pequi is rich in various important components, such as dietary fiber and minerals, including magnesium, iron, zinc and phosphorus (Leão et al., 2017; Silva & Fonseca, 2016). Unsaturated fatty acids, especially oleic acid are the prominent constituents in pequi pulp (Pinto et al., 2018). Additionally, the pequi also contains bioactive compounds such as phytosterols, tocopherols and carotenoids (Silva et al., 2022; Torres et al., 2016), whose consumption is directly linked with several health benefits (Carvalho & Conte-Junior, 2021). The bioactive compounds present in pequi are related with some beneficial biological properties, such as, antioxidant, anticarnogenic, wound-healing and anti-inflammatory activities (Brito et al., 2022; Pereira et al., 2020; Torres et al., 2016).

Therefore, considering all the benefits provided by these two ingredients, the present study aimed to evaluate the effect of pequi flour (PF) addition and different cooking methods on the cooking loss, carotenoids, fatty acid composition, volatile profile and sensory characteristics of chickpea meat analogue (CMA) nuggets.

Material and methods

2.1. Materials

Frozen pequi fruits (*Caryocar brasiliense*) without the exocarp (a greenish-brown outer skin) and the white external mesocarp, harvested in 2021, were obtained from two Fruit Pulp Industries-Barra do Garças (Mato Grosso State, Brazil) and Goiânia (Goias State, Brazil). The fruits were then transported to the Pilot Plant in the Laboratory of Post-Harvest of Fruit and Vegetables Fruit of Federal University of Lavras (UFLA, Lavras, Brazil), where the experiment was carried out. All the ingredients were obtained from local markets in Lavras, Brazil.

2.2. Preparation of pequi flour (PF)

Fruits consisted of the internal mesocarp and endocarp (yellowish-orange pulp) and seeds, without any physical injury and uniform in size and color were visually selected, sanitized with sodium hypochlorite solution (100 mg/L) for 15 min and then rinsed in distilled water to remove the residual chlorine. Afterward, they were bleached in a water bath at 80 ^{o}C for 8 min. After 10 min cooling over in an ice bath, the fruits were then manually pulped using the stainless knife and the pulp and seeds were removed. Subsequently, the pulp was dried in an oven with forced air circulation (Solab SL 102 model, Piracicaba, São Paulo, Brazil) at 40^{o}C for 30 h. The dried pulp was first crushed using an industrial blender (Poli model LS-06, Brusque/Brazil), and then in a knife mill (Tecnal T650 model, Piracicaba, Brazil) and passed through a 20 – mesh sieve (0.85 mm) to obtain the pequi flour. The resulted flour was vacuum-packed in polyethylene plastic bags containing approximately 500g and stored frozen at -20 ^{o}C until further use.

2.3. Formulation of chickpea meat analogue (CMA) nuggets.

Five different formulations (Table 1) in three separated batches were prepared in the pilot plant of Laboratory of Meat Science and Technology at Federal University of Lavras. In brief, chickpeas were boiled for 20 min in a pressure cooker after overnight soaking (12h) at room temperature and ground in an electric grinder (BECCARO model PB-22, Rio Claro, Brazil), with 8 mm disc to obtain a pasta. The chickpea pasta was then mixed with all the other ingredients and additives. The final mixture was molded in a hamburger mold (11cm diameter), immediately frozen at -18 ^{o}C for 8h, cut into four equal pieces (2.5 x 2.5 x 1.0 cm) and refrozen for 1h. After this, the formulated CMA nuggets were predusted with wheat flour, batter-coated

with a mixture comprised of wheat flour, corn starch, salt and ice water, and breaded with dried bread crumbs. After coating, the CMA nuggets were pre-fried in soybean oil at 90 ºC for 30 s, immediately removed from the fryer and drained on absorbent papers at room temperature for 10min to remove the excess surface oil. Finally, the CMA nuggets samples were stored in polyethylene plastic bags and frozen at -18 ºC for 45 days. The physicochemical analyses were carried out at 0, 15, 30 and 45 days of frozen time and the sensory analysis was carried out only at first day of storage period.

Table 1 Formulations of the chickpea meat analogue (CMA) nuggets

Ingredients (%)	Treatments				
	PF0	PF3	PF6	PF9	PF12
Chickpea	95.0	92.0	89.0	86.0	83.0
Pequi flour	0.0	3.0	6.0	9.0	12.0
Salt (NaCl)	1.0	1.0	1.0	1.0	1.0
Phosphate	0.5	0.5	0.5	0.5	0.5
Textured soy protein	1.4	1.4	1.4	1.4	1.4
Sodium erythorbate	0.3	0.3	0.3	0.3	0.3
Garlic powder	0.3	0.3	0.3	0.3	0.3
Onion powder	0.3	0.3	0.3	0.3	0.3
White pepper powder	0.1	0.1	0.1	0.1	0.1
Nutmeg powder	0.1	0.1	0.1	0.1	0.1

PF0, CMA nuggets without pequi flour (control); PF3, PF6, PF9 and PF12, CMA nuggets with 3, 6, 9 and 12% pequi flour, respectively.

2.4. Heat treatments of chickpea meat analogues (CMA) nuggets

Frozen pre-fried chicken nuggets samples were thawed at room temperature and exposed to different heat treatments as follow: (1) Deep frying: the CMA nuggets were fried using 2.5 L soybean oil in a pre-heated domestic deep fryer at 180 ^{o}C for 4 min; the samples were turned over every minute. (2) Air-frying: the samples were placed into a pre-heated air-fryer (Mondial model AF-34, Brazil) at 180 ^{o}C and air-fried for 15 min. (3) Oven-roasting: CMA nuggets were roasted in a pre-heated commercial oven at 150 ^{o}C for 8 min. (4) Microwave: the CMA nuggets samples were placed into a microwave (Brastemap ative, model BMS45B, Brazil) container and cooked at 50% power (410W) for 4 min. The heat temperatures were chosen based on the preliminary experiments. After cooking, all samples were cooled down to the room temperature and used for the analyses.

2.5. Experimental design

A 5 x 4 factorial design, with five levels of pequi flour (0%, 3%, 6%, 9%, 12%) and four levels of cooking methods (air-frying, deep-frying, oven roasting, and microwave) was performed for cooking loss and total carotenoids. Data from fatty acid profile and volatile compounds were evaluated using a 3 x 4 factorial arrangement, with three levels of pequi flour (0%, 6%, 12%) and four levels of cooking methods (air-frying, deep-frying, oven roasting, and microwave). The sensory data were evaluated using completely randomized block design, including assessors as a random effect (repetitions).

2.6. Analyses

2.6.1. Cooking loss

The cooking loss was determined by the difference between the mass of raw nuggets and the fried nuggets multiplied by 100 following equation (1).

$$\text{Cooking loss (\%)} = \left(\frac{\text{Weight before frying - Weight after frying}}{\text{Weight before frying}} \right) x\ 100 \quad (1)$$

2.6.2. Extraction and determination of total carotenoids

Total carotenoids of CMA nuggets were extracted and determined using the method provided by Rodriguez-Amaya (2001) with few changes. In brief, 2.5g of the samples were homogenized with 20 mL of cold acetone and vigorous shaking on a shaker (Nova ethics model

109-2TCM, Vargem Grande Paulista, Brazil) for 20 min. The residue was separated from the liquid phase by filtration using a filter paper with 14-µm porosity and washed three times with acetone (20 mL). The filtrate was transferred into a 250 mL separatory funnel to wich 30 mL of petroleum ether and 100 mL of water were added. After phase separation, the lower phase containing acetone and water was discarded and the washing procedure was repeated three time. The resulting total petroleum ether layer was filtered using a filter paper with 14-µm porosity and the volume was completed to 50 mL with petroleum ether. The absorbances were measured using a UV-VIS spectrophotometer at 444, 450, 456, 462, and 470 nm for α-carotene, β-carotene, δ-carotene, γ-carotene, and lycopene, respectively. The results were expressed in µg/100g of the total carotenoids content using the following equation (2).

$$C = \frac{OD \times V \times 10^6}{A^{1cm1\%} \times W} \times 100 \quad (2)$$

Where, C is the total carotenoid content (µg/100g); OD is the absorbance at each specific wavelength (444, 450, 456, 462, and 470nm); V is the total volume of sample extract solution (mL); $A^{1cm1\%}$ is the specific absorption coefficient of particular carotenoids (α-carotene, β-carotene, δ-carotene, γ-carotene, and lycopene); W is the weight of the sample (g).

2.6.3. Fatty acid profile analysis

The total fat extraction was based on the method described by Bligh & Dyer (1959). Briefly, 4g of CMA nuggets were extracted using methanol, chloroform and water (1:1:2:0.8, v/v/v) mixture and determined by gravimetry in an oven with forced air circulation (Solab SL 102 model, Piracicaba, São Paulo, Brazil) at 105 ºC. Then, 20 mg of fatty acid were converted to fatty acid methyl esters (FAME) by the derivatization reaction proposed by Hartman & Lago (1973). The lipid fraction was added 1mL of methanolic potassium hydroxide solution (0.4 M) and the whole was subjected to a boiling point water bath for 10 min. After cooling, 3 mL of methanolic sulfuric acid solution (1 M) was added and again subjected to heating for 10 min. Finally, 2 mL of hexane were added to solubilize the FAME. A fused silica capillary column

The hexane-diluted FAMEs were quantified by gas chromatography (Shimadzu GC 2010) equipped with a fused silica capillary column (Supelco SP-2560, Bellefonte, PA, EUA; 100 m x 0.25 mm x 0.25 µm film thickness), flame ionization detector and injector split ratio was 1:50. The injector and detector temperatures were 260 ºC. Helium was used as the carrier at a constant flow of 0.8mL/min. The operating column conditions were the following: initial

temperature of 140°C/5 min, which increased to 240 °C at a rate of 4°C/min and it remained at this temperature for 30 min for a total run time of 60 min. The fatty acids peaks were identified by comparison with standards available FAME mixtures (37-component FAME Mix; Supelco Inc., Bellefonte, PA, USA) and the results were expressed as percentage of the total detected FA methyl esters (FAME). The atherogenicity index (AI) and thrombogenicity index (TI) were determined according to equations (3) and (4), developed by Ulbricht & Southgate (1991).

$$AI = (C12:0 + 4 \times C14:0 + C16:0) / \left(\sum MUFA + \sum n\text{-}6 + \sum n\text{-}3\right) \quad (3)$$

$$TI = (C14:0 + C16:0 + C18:0) / \left[\left(0.5 \times \sum MUFA\right) + \left(0.5 \times \sum n\text{-}6\right) + \left(3 \times \sum n\text{-}3\right) + \left(\sum n\text{-}3 / \sum n\text{-}6\right)\right] \quad (4)$$

2.6.4. Volatile compounds analysis

The analysis of volatile compounds from all treatments was performed using the headspace-solid phase microextraction (HS-SPME) technique in accordance with the procedure of Gomide et al. (2022), with few modifications. Briefly, 5 g of CMA nuggets were placed in a 10 mL sealed glass vial with a PTFE-silicone septum (Supelco, Bellefonte, PA, USA). Divinylbenzene/Carboxen/Polydimethylsiloxane (50-30µm coating thickness; 1 cm length; Supelco, Bellefonte, PA, USA) fiber was used for the extraction of volatile compounds. Prior to extraction, the fiber was conditioned at 220 °C for 5 min according to manufacturer instructions.

The samples were incubated at 40 °C for 10 min and then extracted at 40 °C for 30 min, with a constant desorption of 2 min. After extraction, the volatile compounds were separated and identified using a GC-MS QP2010 plus (Shimadzu Corporation, Japan) equipped with an AOC-5000 automatic injector for liquids and gases (Shimadzu, Japan) and an SLBTM column (30 m x 0.25 mm x 0.25 µm 5% phenyl, 95% dimethylsiloxane film. The CG conditions were as follows: the injector temperature was maintained at 220 °C under splitless mode; high purity helium (99.999%) was used as carrier gas, and the flow rate was kept constant at 1.0 mL/min.

The initial oven temperature was set at 40 °C for 4 min, then increased to 80 °C at a rate of 2 °C/min. After, the temperature was increased from 80 °C to 140 °C at a rate of 4 °C/min, and finally increased further for 200 °C at a rate of 8 °C/min, where it was maintained until the end. The mass spectrometer was operated under electron impact mode (70 eV) with a full scan mode from 45 to 60 Da and solvent cut-off in 0.55 min. The interface and ion source temperatures were 240 °C and 200 °C, respectively. Volatile compounds identification was

made by comparing their mass spectra with those of the database (NIST 8, Willey 8 and FFNSC 12) and comparing the retention indices (RI) obtained from the retention time (RT) of homologous series of n-alkanes (C8-C20) with the available literature data. The volatile compounds were reported as relative peak areas (peak area of each compound/total area) x 100.

2.6.5. Sensory analysis

The sensory analysis was carried out at the sensory analysis laboratory of the Federal University of Lavras. Prior to analysis, the research was approved by the institutional ethics commission (code of ethics: CAAE: 55582822.6.0000.5148) and the informed consent was obtained from the panelists. A panel of 17 judges (focus group) of both genders, ages between 18 and 50 years was chosen based on their interest, availability and habit in consuming vegetarian food, at least once a month. The acceptance test using a structured 9-point hedonic scale (1 = dislike very much; 9 = like very much) was used to investigate the effect of different cooking methods on the acceptance of the CMA nuggets.

The panelists evaluated the samples in terms of appearance, aroma, taste, texture, and overall acceptance. The samples consisted only of control CMA nuggets (without pequi) subjected to four thermal processing methods, including air-frying, deep-frying, oven-roasting and microwaving cooked under conditions described in the section 2.4. After cooking, the samples were cut into cubes of 2 cm edge, coded with random three digits numbers and served to consumers in a monadic order. Room temperature water was used to clean the palate between each evaluation. Based on the scores attributed by each panelist and the consensus of the focus group discussion the best method was chosen.

2.7. Statistical analysis

Data were reported as mean ± standard deviation. Analysis of variance (ANOVA) was carried out using R version 4.0.4 (R Core Team, 2020). Significant differences between means for cooking loss, total carotenoids contents and sensory attributes were identified using Tukey's test. Differences among mean for volatile compounds and fatty acids profile were identified using Duncan's multiple range tests. Principal component analysis (PCA) and hierarchical cluster analysis were conducted to visualize the discrimination of the treatments on the volatile compounds. The PCA and hierarchical cluster analysis were performed using Origin Pro 8.0 (OriginLab, USA). For all analyses, the significance level was fixed at $p < 0.05$.

3. Results and discussion

3.1. Cooking loss

Cooking loss (CL) is one of the main cooking parameters directly related to the water holding capacity (WHC) of samples (Zhang et al., 2022). As shown in Fig. 1A, the CL values were significantly ($p < 0.05$) affected by the interaction of both pequi flour (PF) concentrations and cooking method. Compared with the control samples, the CL gradually decreased with increasing the amount of PF for all treatments. This may be due to synergistic effect of the dietary fiber from chickpea and PF, which led to higher water retention. Other researchers have also reported the reduced CL in plant-based meat analogue patties formulated with dietary fiber such as garlic mushroom powder, gelatin, and methylcellulose (Bakhsh et al., 2021, 2022). Additionally, Sakai et al. (2022), reported that the addition of natural colorants like laccase, sugar beet pectin protein, and beet red pigments in plant-based meat patties lowered the CL and enhanced the WHC.

The air-fried samples resulted in a significantly higher ($p < 0.05$) CL than the other cooking methods, with the lowest loss being found for deep-oil fried samples, regardless of PF level. Similar to our outcomes, Kwon et al. (2023) recently reported the higher CL in chicken wing and pork belly cooked by air-frying at 140 ^{o}C than that cooked by pan frying. The higher CL of air-fried samples and the lower CL of deep-oil fried samples among the other cooking methods could be explained by the higher cooking time (15 min) of air-frying and the lower cooking time (4 min) of deep-oil frying.

3.2. Total carotenoids

Carotenoids are one of the most important colored pigments commonly found in food and are considered to have a functional role against several diseases (Mehmood & Zeb, 2020). As shown in Fig. 1B, the interaction of PF with cooking method displayed a significant impact on the total carotenoid contents ($p < 0.05$). As expected, compared to control samples, regardless the cooking method, the total carotenoids contents in CMA nuggets significantly ($p < 0.05$) increased as the proportion level of PF increased, due to the presence of PF that has high level of carotenoids compared with chickpeas. Overall, the highest retentions of carotenoids were recorded in microwaved and air-fried samples, while the lowest retentions were recorded in deep-oil fried CMA nuggets.

The present results suggested that microwave and air-frying are the best cooking method for the preservation and retention of carotenoids. Carotenoids are very susceptible to degradation by chemical and physical factors such as UV light and oxygen levels, pH changes, and high temperature (Boon et al., 2010). Thus, in the present study, the differences in the total carotenoids contents among the cooking methods might be associated with the effect of heat generated during cooking, cooking conditions, cooking time, as well as temperature resulting in different chemical compositions.

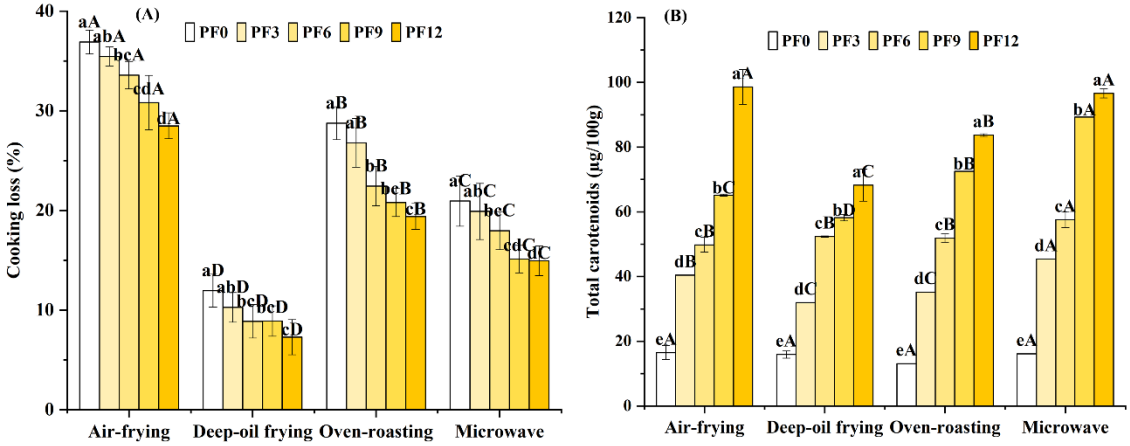

Fig.1. Effect of pequi flour and cooking methods on cooking loss (A) and total carotenoids (B); CMA: chickpea meat analogue; PF: pequi flour; PF0: control CMA nuggets (without PF); PF6 and PF12: CMA nuggets with 6 and 12% of PF incorporation, respectively. Different letters (A-D) indicate significant differences between different levels of pequi flour (bars with different colors) for the same cooking method ($p < 0.05$), while different letters (a-c) indicate significant differences between different cooking methods for the same level of pequi flour (bars with the same color) ($p < 0.05$) by Tukey test.

3.3. Fatty acid profile

The fatty acid profile of raw and cooked CMA nuggets is given in the Table 1. In raw samples a total of 13 fatty acids were detected while in the cooked samples 14 fatty acids were detected. In all samples the main fatty acids from high to low were linoleic acid (C18:2n6c), oleic acid (C18:1n9c) and palmitic acid (C16:0) of the polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA), respectively. The fatty acid profile of the CMA nuggets was significantly ($p < 0.05$) affected by the incorporation of PF and cooking method.

In raw CMA nuggets, increasing the PF level significantly increased the oleic acid (C18:1n9c) and palmitic acid (C16:0) and reduced the linoleic acid (C18:2n6c) and linolenic

acid (C18:3n3). The increment in the contents of oleic and palmitic acids in the raw samples might be related to the high level of these fatty acids present in pequi pulp (Nascimento-Silva & Naves, 2019). The results also indicated that the SFA and MUFA contents of raw CMA nuggets increased significantly ($p < 0.05$) with the increasing level of PF, whereas the PUFA contents were found to decrease. The increased of SFA and MUFA with the increase of PF in the raw samples may be due to the increase of oleic and palmitic acids, respectively. On the other hand, the reduction of PUFA values may be due to the decrease of linoleic acid. Several authors have highlighted that PUFA consisted predominantly of linoleic (C18:2) fatty acid, whereas MUFA consisted mainly of oleic (C18:1) fatty acid (Abdel-Naeem et al., 2021; Matics et al., 2017).

Deep-oil frying led to a significant ($p < 0.05$) higher PUFA, along with a significant ($p < 0.05$) lower MUFA and SFA in comparison with the raw samples. However, in general, there were no significant differences in PUFA, MUFA, and SFA in CMA nuggets cooked by air-frying, oven-roasting and microwave. The high PUFA in the deep-oil fried samples compared to the other cooking methods may be due to the significant ($p < 0.05$) higher linoleic acid (C18:2n6c) concentration as consequence of cooking. On the other hand, the changes in MUFA and SFA may be due to the significant ($p < 0.05$) lower oleic acid (C18:1n9c) and palmitic acid (C16:0) levels, respectively, during the deep-oil frying process. PUFA/SFA ratio in the raw CMA nuggets decreased substantially ($p < 0.05$) with the incorporation of PF, due to both, the reduction in PUFA and the increase in SFA contents. In cooked samples, the PUFA/SFA ratio also reduced significantly; however, all cooking methods used in this study, provided the PUFA/SFA ratio within the minimum recommended ratio (> 0.4) to reduce the incidence of cardiovascular diseases (FAO/WHO, 2010).

The n-6/n-3 ratio is considered an important index to assess the lipid quality and it can help to prevent the emergence of several diseases (Abdel-Naeem et al., 2021). The ratio of 2.5 to 8:1 is recommended for a good lipid quality product (WHO/FAO, 2003). A ratio of 10:1 has been proposed for n-6/n-3 to prevent the risks of obesity, chronic diseases and cardiovascular diseases (Simopoulos, 2016). In the raw samples, no significant differences ($p > 0.05$) were observed in the n-6/n-3 index with the increase of PF. Regarding the cooking methods, the results showed that in general, there were no obvious differences in n-6/n-3 ratio in samples that were cooked by air-frying, oven-roasting and microwave compared to raw samples; however, the deep-oil fried CMA nuggets, due to the high content of n-3 in comparison with the other cooking methods, had significantly ($p < 0.05$) lower n-6/n-3 ratio which was almost

within the recommended limit (10:1) to prevent the incidence of obesity, chronic diseases and cardiovascular diseases.

The atherogenic index (AI) and thrombogenic index (TI) are crucial tools that have been suggested as good indicators of healthy products and have been widely calculated and used to estimating the chance of food to develop coronary heart diseases. Besides, they also provide the information about the nutritional quality of lipids, where low values of these indices indicating healthier food with good nutritional quality of fatty acids and subsequent higher potential for avoiding the emergence of the coronary heart diseases (Botella-Martinez et al., 2021; Weber et al., 2008). In the raw CMA nuggets, as the incorporation level increased, the AI and TI increased significantly ($p < 0.05$). All the cooking methods induced significant ($p < 0.05$) higher AI and TI except deep-oil frying which yielded a slightly lower AI and TI values, as compared with the raw samples. However, it is important to highlight that all the cooking methods showed AI and TI values within the threshold limit (< 1.0) for good lipid quality of the product (Di Bella et al., 2022).

Table 2 Effects of pequi flour (PF) and cooking methods on the fatty acids profile, ratios and health indexes of chickpea meat analogue (CMA) nuggets

Cooking methods	Raw			Air-frying			Deep-frying			Oven-roasting			Microwave		
Pequi flour (%)	0	6	12	0	6	12	0	6	12	0	6	12	0	6	12
Fatty acid composition (%)															
C4:0	/	/	/	0.30^{aA}	0.21^{aB}	0.08^{bC}	0.16^{bA}	0.15^{aA}	0.19^{aA}	0.35^{aA}	0.18^{aB}	0.22^{aB}	0.18^{bA}	0.18^{aA}	/
C14:0	0.26^{aA}	0.30^{bA}	0.27^{aA}	0.25^{aA}	0.23^{bA}	0.25^{aA}	0.15^{bA}	0.14^{cA}	0.15^{bA}	0.22^{abA}	0.23^{bA}	0.23^{aA}	0.23^{aB}	0.40^{aA}	0.21^{abB}
C16:0	17.57^{aC}	21.88^{bB}	24.12^{bA}	11.77^{cC}	23.01^{bB}	26.84^{aA}	13.93^{bC}	16.97^{cB}	19.63^{cA}	14.88^{bC}	21.94^{bB}	25.52^{abA}	11.95^{cB}	25.90^{aA}	25.38^{abA}
C16:1	0.33^{aA}	0.40^{bA}	0.43^{aA}	0.15^{aA}	0.36^{bA}	0.41^{aA}	0.24^{aA}	0.21^{bA}	0.25^{aA}	0.20^{aA}	0.36^{bA}	0.41^{aA}	0.13^{aC}	1.12^{aA}	0.40^{aB}
C17:0	0.07^{bA}	0.08^{bA}	0.08^{bA}	0.08^{abA}	0.08^{abA}	0.08^{abA}	0.09^{aA}	0.08^{aA}	0.08^{aA}	0.08^{abA}	0.07^{abA}	0.08^{abA}	0.10^{aA}	0.09^{aA}	0.08^{aA}
C18:0	2.93^{bA}	2.52^{bB}	2.32^{cB}	3.10^{bA}	2.68^{bA}	2.72^{bA}	3.91^{aA}	3.90^{aA}	3.77^{aA}	3.29^{bA}	2.70^{bB}	2.77^{bB}	3.67^{aA}	3.80^{aA}	2.91^{bB}
C18:1n9c	32.80^{aB}	37.51^{bA}	39.47^{bA}	26.04^{cC}	37.63^{bB}	43.04^{aA}	28.20^{bcB}	30.30^{cB}	34.23^{cA}	29.10^{bC}	36.34^{bB}	42.82^{aA}	25.96^{cB}	41.96^{aA}	41.56^{abA}
C18:2n6c	41.55^{cA}	33.56^{cB}	30.22^{bC}	52.98^{aA}	32.46^{cB}	23.87^{dC}	47.43^{bA}	43.01^{aB}	37.04^{aC}	47.06^{bA}	34.71^{bB}	25.53^{cC}	52.08^{aA}	33.06^{cB}	26.20^{cC}
C20:0	0.38^{aA}	0.33^{bcB}	0.33^{bB}	0.49^{bA}	0.41^{aB}	0.35^{bC}	0.47^{bA}	0.44^{aAB}	0.41^{aB}	0.49^{bA}	0.36^{bB}	0.33^{bB}	0.51^{bA}	0.30^{cC}	0.35^{bB}
C18:3n6	0.16^{cA}	0.11^{bB}	0.08^{bC}	0.17^{cA}	0.08^{cB}	0.08^{bB}	0.25^{aA}	0.22^{aB}	0.20^{aB}	0.16^{cA}	0.12^{bB}	0.08^{bC}	0.22^{bA}	0.10^{bcB}	0.10^{bB}
C20:1	0.21^{aA}	0.22^{bA}	0.23^{aA}	0.23^{aA}	0.24^{aA}	0.23^{abA}	0.22^{aA}	0.18^{cB}	0.17^{cB}	0.23^{aA}	0.21^{bA}	0.21^{abA}	0.21^{aA}	0.22^{abA}	0.20^{bA}
C18:3n3	3.36^{cA}	2.56^{bB}	2.06^{bC}	3.83^{bA}	2.22^{bB}	1.76^{bC}	4.31^{aA}	3.71^{aB}	3.45^{aB}	3.33^{cA}	2.52^{bB}	2.11^{bC}	4.01^{abA}	1.81^{cB}	2.0^{8b}
C22:0	0.31^{cA}	0.26^{cB}	0.24^{bB}	0.44^{bA}	0.31^{bB}	0.25^{bC}	0.47^{abA}	0.45^{aA}	0.38^{aB}	0.45^{bA}	0.31^{bB}	0.23^{bC}	0.51^{aA}	0.22^{cB}	0.26^{bB}
C22:2	0.16^{bA}	0.15^{cA}	0.17^{aA}	0.23^{aA}	0.20^{aB}	0.18^{aC}	0.23^{aA}	0.20^{aB}	0.19^{aB}	0.24^{aA}	0.17^{bcB}	0.17^{aB}	0.24^{aA}	0.19^{abB}	0.17^{aB}
Nutritional index															
Σ SFA	21.52^{aB}	25.36^{bA}	27.36^{bA}	16.43^{dC}	26.93^{bB}	30.56^{aA}	19.18^{bcC}	22.14^{cB}	24.61^{cA}	19.76^{abC}	25.79^{bB}	29.36^{abA}	17.15^{cdB}	30.88^{aA}	29.18^{abA}
Σ MUFA	33.34^{aB}	38.13^{bA}	40.13^{bA}	26.43^{bC}	38.24^{bB}	43.69^{aA}	28.66^{bB}	30.69^{cB}	34.65^{cA}	29.53^{bC}	36.91^{bB}	43.43^{aA}	26.30^{bB}	43.31^{aA}	42.16^{abA}
Σ PUFA	45.23^{cA}	36.39^{bcB}	32.52^{bC}	57.21^{aA}	34.97^{cB}	25.89^{dC}	52.22^{bA}	47.14^{aB}	40.88^{aC}	50.78^{bA}	37.53^{bB}	27.89^{cC}	56.55^{aA}	35.15^{cB}	28.55^{cC}
ΣPUFA/ΣSFA	2.10^{eA}	1.44^{bB}	1.19^{bC}	3.48^{aA}	1.30^{cB}	0.85^{cC}	2.72^{cA}	2.13^{aB}	1.66^{aC}	2.57^{dA}	1.46^{bB}	0.95^{cC}	3.30^{bA}	1.16^{dB}	0.99^{cC}
Σ n-3	3.36^{cA}	2.56^{bB}	2.06^{bC}	3.83^{bA}	2.22^{bB}	1.76^{bC}	4.31^{aA}	3.71^{aB}	3.45^{aB}	3.33^{cA}	2.52^{bB}	2.11^{bC}	4.01^{abA}	1.81^{cB}	2.08^{bB}
Σ n-6	41.71^{cA}	33.67^{cB}	30.29^{bC}	53.15^{aA}	32.54^{cB}	23.95^{dC}	47.67^{bA}	43.23^{aB}	37.24^{aC}	47.21^{bA}	34.83^{bB}	25.61^{cC}	52.29^{aA}	33.16^{cB}	26.29^{cC}
n-6/n-3	12.42^{abA}	13.18^{abA}	14.78^{abA}	13.89^{aA}	14.69^{aA}	13.61^{aA}	11.07^{bA}	11.64^{bA}	10.79^{bA}	14.19^{abA}	13.81^{abA}	12.17^{abA}	13.04^{aA}	21.18^{aA}	12.65^{aA}
AI	0.24^{aC}	0.31^{bcB}	0.35^{cA}	0.15^{dC}	0.33^{bB}	0.40^{aA}	0.18^{cC}	0.23^{dB}	0.27^{dA}	0.20^{bC}	0.31^{cB}	0.37^{bA}	0.16^{dC}	0.35^{aB}	0.37^{bA}
TI	0.44^{aC}	0.57^{bB}	0.64^{cA}	0.29^{dC}	0.62^{bB}	0.76^{aA}	0.35^{bcC}	0.44^{cB}	0.51^{dA}	0.38^{bC}	0.57^{bB}	0.70^{bA}	0.31^{cdB}	0.69^{aA}	0.70^{bA}

Data were presented as mean of three replicates (n = 3). CMA: chickpea meat analogue; PF: pequi flour; PF0: control CMA nuggets (without PF); PF6 and PF12: CMA nuggets with 6 and 12% of PF incorporation, respectively. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; AI: atherogenicity index; TI: thrombogenicity index. Different letters (A-C) indicate significant differences

between different levels of pequi flour for the same cooking method ($p < 0.05$), while different letters (a-e) indicate significant differences between different cooking methods for the same level of pequi flour ($p < 0.05$) by Duncan's test. " / "- not detected.

3.4. Volatile compounds (VCs)

Food flavor is one of the most important factors influencing the consumer acceptance. As shown in the Table 2, a total of 47 VCs were identified in raw and cooked CMA nuggets belonging to six chemical classes, including terpenoids (13), esters (13), alcohols (9), aldehydes (6), aliphatic hydrocarbons (4), and ketones (2). Terpenoids, were the dominant chemical classes, followed by esters and alcohols. (Z)-β-Ocimene, ethyl hexanoate, terpinene-4-ol and hexanal of the terpenoids, esters, alcohols, and aldehydes, respectively, were the most representative VCs in the raw CMA nuggets. As expected, significant differences ($p < 0.05$) were observed in the VCs of CMA nuggets depending on both, the concentrations of PF (0%, 6%, 12%) and the cooking method used (air-frying, deep-oil frying, oven-roasting and microwave).

There was a significant ($p < 0.05$) interaction between the addition of PF and cooking methods. In the raw samples, it was noted that the inclusion of PF caused a significant ($p < 0.05$) increase of total terpenoids and esters, besides a significant ($p < 0.05$) reduction in the percentages of alcohols, aldehydes and total aliphatic hydrocarbons. However, the ketones were totally absent in the raw samples. The possible reason for the increase percentage of terpenoids and esters could be the increase of (Z)-β-Ocimene and ethyl hexanoate, respectively. The incorporation of spices in the CMA nuggets might also induced the increase of terpenoids, mainly (Z)-β-Ocimene (Yao et al., 2022). With regard the esters, it has been reported that ethyl hexanoate was one of the most dominant VC in pequi pulp (Belo et al., 2013; Damiani et al., 2009).

Independent of the cooking methods used, the findings showed that in general, there was a significant increase of terpenoids, with microwaved samples having the higher percentages of terpenoids when compared with raw and other cooking methods. However, some terpenoids including sabinene, 2-β-pinene, m-cymene and β-elemene wich were found in the raw samples were not detected after cooking. The increase of terpenoids in all cooking methods might be related to the degradation of carotenoids by heating. Yao et al., (2022) reported that carotenoids are thermal degraded by high temperature to generate terpenes and ketones. In addition, it has been reported that terpenoids are mainly generated during the oxidation and degradation of fatty acids (Zhang et al., 2023). In other hand, the differences observed among the treatments could be related to the efficacy of heat transfer depending on the cooking method (Lee et al., 2021).

Compared with the raw CMA nuggets, the concentrations of esters decreased significantly ($p < 0.05$), regardless of cooking conditions; however, deep-fried samples induced higher percentages of esters than the others cooking methods. Some esters such as amyl formate, ethyl butanoate, ethyl 2-methylbutyrate, isobutyl isovalerate, cis-sabinene hydrate acetate, 3-methylbutanoic acid 3-methylbutyl ester and isopropyl 3-methylbutanoate, which were detected in raw samples, were not found in cooked samples. The decrease of esters after CMA nuggets cooking might be related to their volatilization at high temperature and the extension of cooking time (Wang et al., 2021). In addition, heating during the cooking process could have accelerated the reactions of CMA nuggets constituents which might induced the degradation of the heat-sensitive unstable esters, leading to alcoholysis, ammonolysis, ester exchange and reductions reactions to generate new VCs (Xu et al., 2022). Alcohols are mainly derived by the decomposition products of lipid oxidation. However, they have high odor thresholds, which contribute little to the flavor of cooked products (Domínguez et al., 2019). Regardless of the cooking methods used, the results showed that in general, the levels of alcohols decreased significantly ($p < 0.05$) due to the degradation of alcohols into corresponding esters, acids and aldehydes (Sun et al., 2010). Some alcohols including 1-heptanol, 1-pentanol and 2-propen-1-ol not detected in raw samples were generated in the cooked samples. On the other hand, 3-methyl-1-butanol, 2-methyl-1-butanol, 1-hexanol and 2-methylpentanol were only detected in the raw CMA nuggets.

All the cooking methods resulted in the increase of aldehydes contents; however, in general, deep-oil frying induced lower contents of aldehydes than air-fried, oven-roasting and microwave. The increase of aldehydes might be related to the lipid oxidation. It has been reported that aldehydes were the main odor compounds of high-fat cooked products due to their strong volatility and high content and low thresholds (Shi et al., 2019). Previous studies have demonstrated that aldehydes are mostly derived from the decomposition products of lipid oxidation (Yang et al., 2017). The most dominant aldehydes (hexanal, 2-ethylhexanal and pentanal) were derived from the thermal degradation and oxidation of fat (Wang et al., 2021). Hexanal, 2-ethylhexanal and pentanal are mostly generated from the oxidation of linoleic acid and arachidonic acid (Watanabe et al., 2015).

Aliphatic hydrocarbons showed a trend of decreasing as the proportion level of PF in the raw samples increased, and only two compounds (dodecamethyl-cyclohexasiloxane and hexane) were detected. However, the content of aliphatic hydrocarbons in the cooked samples increased, being oven-roasting and deep-oil frying with the highest levels of these compounds

when compared with other cooking methods. The increase of aliphatic hydrocarbons can be attributed to the automatic oxidation of long-chain fatty acids or thermal degradation of lipids (Perez-Santaescolastica et al., 2022). Although the levels of aliphatic hydrocarbons increased in the cooked samples, they had a limited contribution on the flavor of food because of their high odor detection threshold (Qiu et al., 2023).

Ketones were not detected in the raw samples with the incorporation of PF; however, in the cooked samples their amounts increased significantly ($p < 0.05$), being air-frying and oven-roasting, in general, with the higher contents. Previous studies reported that ketones resulted mostly from oxidative decomposition of lipid in high fat products (Roldán et al., 2015). Moreover, thermal treatments might also have contributed to the increase of ketones contents.

Table 3 Effects of flour (PF) and cooking methods on the volatile compounds of chickpea meat analogue (CMA) nuggets

Cooking methods		Raw			Air-frying			Deep-frying			Oven-roasting			Microwave		
Pequi flour (%)		PF0	PF6	PF12	PF0	PF6	PF12	PF0	PF6	PF12	PF0	PF6	PF12	PF0	PF6	PF12
Compounds																
Terpenoids	**RI**															
α-Terpinene	1016	0.50^{cA}	0.18^{aB}	/	0.79^{bA}	0.25^{aB}	0.19^{aB}	1.24^{aA}	0.20^{aB}	0.10^{aB}	0.66^{bcA}	0.24^{aB}	/	/	0.19^{aA}	/
α-Thujene	932	0.82^{dA}	0.28^{aB}	0.11^{aB}	2.26^{bA}	0.57^{aB}	0.37^{aB}	3.01^{aA}	0.29^{aB}	/	1.85^{cA}	0.53^{aB}	0.24^{aC}	2.94^{aA}	0.52^{aB}	0.21^{aC}
β-Phellandrene	971	/	/	/	1.63^{cA}	0.50^{aB}	0.34^{aB}	2.78^{aA}	0.38^{aB}	0.17^{abC}	1.64^{cA}	0.47^{aB}	0.30^{aB}	1.85^{bA}	0.47^{aB}	0.20^{aC}
γ-Terpinene	1057	1.38^{dA}	0.62^{aB}	0.28^{aC}	2.13^{bA}	0.78^{aB}	0.54^{aB}	2.94^{aA}	0.68^{aB}	0.35^{aC}	1.77^{cA}	0.76^{aB}	0.37^{aC}	1.81^{cA}	0.74^{aB}	0.38^{aC}
(E)-β-Ocimene	1034	2.07^{aB}	3.25^{bA}	3.32^{bcA}	/	3.47^{abA}	2.99^{cB}	/	3.32^{bA}	3.45^{abA}	2.37^{aB}	3.61^{abA}	3.46^{abA}	/	3.82^{aA}	3.75^{aA}
(Z)-β-Ocimene	1045	32.68^{aB}	43.13^{bA}	39.94^{bA}	13.34^{bC}	43.62^{bA}	35.32^{cB}	8.07^{cC}	45.10^{bA}	41.68^{bB}	35.89^{aC}	46.60^{bA}	41.97^{bB}	8.39^{cC}	53.04^{aA}	46.82^{aB}
β-Pinene	977	/	/	/	2.11^{cA}	0.55^{aB}	0.39^{aC}	3.30^{bA}	0.34^{bB}	/	1.99^{cA}	0.59^{aB}	0.27^{aC}	3.78^{aA}	0.61^{aB}	0.27^{aC}
Limonene	1028	6.07^{cA}	5.65^{cA}	4.45^{bB}	19.03^{aA}	12.38^{aB}	7.42^{aC}	3.62^{dB}	5.31^{cA}	2.92^{cB}	5.88^{cA}	5.76^{cA}	3.01^{cB}	15.19^{bA}	7.12^{bB}	4.53^{bC}
Myrcene	987	0.54^{bB}	0.68^{cdA}	0.70^{aA}	1.02^{aA}	0.96^{aA}	0.65^{abB}	/	0.62^{dA}	0.59^{bA}	0.56^{bB}	0.69^{bcA}	0.62^{bB}	0.46^{cB}	0.76^{bA}	0.72^{aA}
Sabinene	971	0.98^{aA}	0.35^{aB}	0.13^{aC}	/	/	/	/	/	/	/	/	/	/	/	/
2-β-Pinene	987	0.87aA	0.28aB	/	/	/	/	/	/	/	/	/	/	/	/	/
M-Cymene	1023	1.12^{aA}	0.39^{aB}	/	/	/	/	/	/	/	/	/	/	/	/	/
β-elemene	1423	0.27^{aA}	0.06^{aB}	/	/	/	/	/	/	/	/	/	/	/	/	/
Total Terpenoids		**47.30^{bB}**	**54.87^{dA}**	**48.93^{bB}**	**42.31^{cC}**	**63.08^{bA}**	**48.21^{bB}**	**24.96^{eC}**	**56.24^{cdA}**	**49.26^{bB}**	**52.61^{aB}**	**59.25^{cA}**	**50.24^{bB}**	**34.42^{dC}**	**67.27^{aA}**	**56.88^{aB}**
Esters																
Ethyl 2-hexenoate	1042	/	0.46^{aB}	0.57^{aA}	/	0.27^{cA}	0.45^{bB}	/	0.46^{aB}	0.55^{aA}	/	0.30^{bB}	0.42^{bcA}	/	0.24^{cB}	0.41^{cA}
Ethyl isovalerate	849	0.83^{aB}	0.69^{aC}	1.29^{aA}	/	0.14^{cB}	0.29^{cA}	/	0.51^{bA}	0.44^{bA}	/	0.14^{cB}	0.32^{cA}	/	0.15^{cB}	0.37^{bcA}
Ethyl hexanoate	996	22.67^{aC}	27.49^{aB}	34.26^{aA}	7.60^{bcC}	15.63^{cB}	31.11^{abA}	6.26^{cC}	24.60^{aB}	33.33^{aA}	10.04^{bC}	17.87^{bcB}	29.20^{bA}	5.53^{cC}	18.95^{bB}	29.35^{bA}
Ethyl octanoate	1193	2.00^{aC}	4.03^{bB}	5.42^{cA}	/	5.16^{aB}	8.71^{aA}	/	5.16^{aB}	8.65^{aA}	2.31^{aC}	4.84^{aB}	7.12^{bA}	/	5.13^{aB}	7.19^{bA}
Methyl hexanoate	921	1.83^{aA}	1.03^{aC}	1.26^{aB}	/	0.51^{cA}	0.37^{dB}	/	0.63^{bA}	0.54^{bB}	/	/	0.35^{dA}	/	/	0.43^{cA}
Isoamyl acetoacetate	756	/	/	/	/	/	/	2.25^{aA}	1.07^{aB}	0.96^{aC}	/	0.39^{bA}	0.27^{bB}	/	/	/
Amyl formate	776	0.80^{aA}	0.50^{aB}	0.56^{aB}	/	/	/	/	/	/	/	/	/	/	/	/
Ethyl butanoate	800	2.71^{aA}	0.55^{aB}	0.51^{aB}	/	/	/	/	/	/	/	/	/	/	/	/
Ethyl 2-methylbutyrate	845	/	0.28^{aB}	0.60^{aA}	/	/	/	/	/	/	/	/	/	/	/	/
Isobutyl isovalerate	1004	/	0.22^{aB}	0.24^{aA}	/	/	/	/	/	/	/	/	/	/	/	/
Cis-sabinene hydrate acetate	1070	0.76^{aA}	0.24^{aB}	0.10^{aC}	/	/	/	/	/	/	/	/	/	/	/	/
3-Methylbutanoic acid 3-methylbutyl ester	1104	0.79^{aB}	0.94^{aA}	0.81^{aB}	/	/	/	/	/	/	/	/	/	/	/	/
Isopropyl 3-methylbutanoate	891	/	0.12^{aB}	0.19^{aA}	/	/	/	/	/	/	/	/	/	/	/	/
Total Esters		**32.39^{aC}**	**36.55^{aB}**	**45.81^{aA}**	**7.60^{cC}**	**21.71^{cB}**	**40.93^{bA}**	**8.51^{cC}**	**32.43^{bB}**	**44.47^{aA}**	**12.35^{bC}**	**23.54^{cB}**	**37.68^{cA}**	**5.53^{cC}**	**24.47^{cB}**	**37.75^{cA}**

Alcohols																
1-Heptanol	1103	/	/	/	2.02^{bA}	1.26^{aB}	0.85^{aC}	3.90^{aA}	1.28^{aB}	0.59^{bC}	1.34^{dA}	1.17^{abA}	0.68^{bB}	1.58^{cA}	1.02^{bB}	0.67^{bC}
1-Pentanol	777	/	/	/	/	0.35^{cA}	0.25^{cA}	1.88^{aA}	1.23^{aB}	0.94^{aC}	/	0.47^{bcA}	0.44^{bA}	/	0.51^{bA}	0.33^{bcB}
2-Propen-1-ol	721	/	/	/	1.10^{bA}	0.27^{aB}	0.19^{aB}	1.54^{aA}	0.25^{aB}	/	1.16^{bA}	0.39^{aB}	0.21^{aC}	0.51^{cA}	/	/
3-Ethyl-pentan-2-ol	1247	/	0.40^{aA}	0.50^{bA}	/	0.56^{aA}	0.63^{abA}	/	0.45^{aB}	0.76^{aA}	/	0.57^{aA}	0.59^{abA}	/	/	0.68^{abA}
Terpinene-4-ol	1181	4.36^{bA}	1.29^{aB}	0.53^{aB}	4.78^{bA}	1.58^{aB}	1.06^{aB}	8.92^{aA}	1.68^{aB}	0.83^{aB}	3.63^{bA}	1.54^{aB}	0.67^{aB}	3.55^{bA}	1.21^{aB}	0.61^{aB}
3-Methyl-1-butanol	755	2.54^{aA}	1.08^{aB}	0.98^{aB}	/	/	/	/	/	/	/	/	/	/	/	/
2-Methyl-1-butanol	758	0.59^{aA}	0.27^{aB}	0.25^{aB}	/	/	/	/	/	/	/	/	/	/	/	/
1-Hexanol	867	1.08^{aB}	0.63^{aC}	1.22^{aA}	/	/	/	/	/	/	/	/	/	/	/	/
2-Methylpentanol	1054	0.46^{aC}	0.74^{aB}	0.80^{aA}	/	/	/	/	/	/	/	/	/	/	/	/
Total Alcohols		**9.03^{bA}**	**4.41^{aB}**	**4.28^{aB}**	**7.90^{cA}**	**4.02^{aB}**	**2.98^{bC}**	**16.24^{aA}**	**4.89^{aB}**	**3.12^{bC}**	**6.13^{dA}**	**4.14^{aB}**	**2.59^{bC}**	**5.64^{dA}**	**2.74^{bB}**	**2.29^{bB}**
Aldehydes																
3-Methylbutanal	717	/	/	/	0.58^{cA}	0.23^{aB}	0.16^{aB}	0.99^{aA}	0.16^{aB}	/	0.76^{bA}	0.30^{aB}	0.18^{aB}	/	/	/
Hexanal	801	4.95^{dA}	2.08^{bB}	0.05^{cC}	8.06^{cA}	2.30^{abB}	1.47^{aC}	13.10^{aA}	2.10^{bB}	/	4.27^{eA}	2.61^{aB}	0.99^{bC}	8.77^{bA}	2.34^{abB}	0.80^{bC}
2-Ethylhexanal	700	/	/	/	3.28^{cA}	0.55^{abB}	0.38^{aB}	5.94^{bA}	0.42^{abcB}	0.10^{aB}	2.67^{dA}	0.85^{aB}	0.37^{aC}	7.63^{aA}	0.35^{bcB}	0.21^{aB}
Pentanal	734	0.95^{dA}	0.39^{cB}	/	2.05^{bA}	1.27^{aB}	0.75^{bC}	2.49^{aA}	0.38^{cB}	/	1.63^{cA}	1.41^{aB}	0.99^{aC}	2.45^{aA}	0.84^{bB}	0.73^{bB}
3-Methylbutanal	717	0.36^{aA}	0.16^{aB}	/	/	/	/	/	/	/	/	/	/	/	/	/
Nonyl aldehyde	1103	0.96^{aA}	0.75^{aB}	0.42^{aC}	/	/	/	/	/	/	/	/	/	/	/	/
Total Aldehydes		**7.22^{eA}**	**3.37^{cB}**	**0.47^{cC}**	**13.97^{cA}**	**4.35^{bB}**	**2.76^{aC}**	**22.53^{aA}**	**3.06^{cB}**	**0.10^{cC}**	**9.33^{dA}**	**5.18^{aB}**	**2.53^{aC}**	**18.85^{bA}**	**3.52^{cB}**	**1.74^{bC}**
Aliphatic hydrocarbons																
Dodecamethyl-cyclohexasiloxane	1298	2.39^{dA}	0.58^{aB}	0.34^{aB}	6.01^{bA}	1.47^{aB}	1.01^{aB}	8.26^{aA}	1.83^{aB}	1.01^{aC}	5.76^{bcA}	2.00^{aB}	1.69^{aB}	4.66^{cA}	1.11^{aB}	0.52^{aB}
Decamethyl-cyclopentasiloxane	1132	/	/	/	2.00^{aA}	0.35^{aB}	0.32^{bB}	2.47^{aA}	0.57^{aB}	0.38^{abB}	2.51^{aA}	0.71^{aB}	1.04^{aB}	1.03^{bA}	0.31^{aB}	/
3-Ethylpentane	1053	/	/	/	/	0.58^{cB}	0.63^{bA}	/	0.73^{aA}	0.68^{aB}	/	0.61^{bA}	0.56^{cB}	/	0.60^{bcB}	0.65^{bA}
Hexane	700	1.67^{aA}	0.21^{aB}	0.18^{aB}	/	/	/	/	/	/	/	/	/	/	/	/
Total Aliphatic hydrocarbons		**4.07^{cA}**	**0.79^{bB}**	**0.52^{bB}**	**8.01^{bA}**	**2.40^{abB}**	**1.97^{abB}**	**10.73^{aA}**	**3.13^{aB}**	**2.07^{abB}**	**8.26^{bA}**	**3.33^{aB}**	**3.29^{aB}**	**5.68^{cA}**	**2.02^{abB}**	**1.17^{bB}**
Ketones																
2-Propanone	684	/	/	/	20.22^{bA}	4.46^{aB}	2.90^{aC}	17.04^{cA}	/	/	11.31^{dA}	4.57^{aB}	3.39^{aB}	29.88^{aA}	/	/
3-Methyl-4-octanone	940	/	/	/	/	/	0.24^{bA}	/	0.27^{aB}	0.97^{aA}	/	/	0.27^{bA}	/	/	0.17^{bA}
Total Ketones		/	/	/	**20.22^{bA}**	**4.46^{aB}**	**3.14^{aB}**	**17.04^{cA}**	**0.27^{bB}**	**0.97^{bB}**	**11.31^{dA}**	**4.57^{aB}**	**3.66^{aB}**	**29.88^{aA}**	/	**0.17^{bB}**

Data were presented as mean of three replicates (n = 3). CMA: chickpea meat analogue; PF: pequi flour; PF0: control CMA nuggets (without PF); PF6 and PF12: CMA nuggets with 6 and 12% of PF incorporation, respectively. RI: retention index. Different letters (A-C) indicate significant

differences between different levels of pequi flour for the same cooking method ($p < 0.05$), while different letters (a-e) indicate significant differences between different cooking methods for the same level of pequi flour ($p < 0.05$) by Duncan's test. " / "- not detected.

3.4.1. Cluster heatmap analysis

In order to assess the relationship between the volatile compounds (VCs) in raw and cooked CMA nuggets by different methods, cluster heatmap analysis was performed and the samples were clustered using the Euclidean distance and the Ward linkage method (Fig. 2). Columns and rows represent each VC and different treatments groups, respectively. Darker orange indicates higher and darker green indicates lower concentration, respectively. Shorter Euclidean distance indicates similarity of the samples. The results showed that there were similarities among the VCs of CMA nuggets in different treatments groups. The samples were divided into four main clusters (Fig. 2). The first cluster consisted of α-Terpinene, γ-Terpinene, hexanal, terpinene-4-ol, β-Phellandrene, dodecamethyl-cyclohexasiloxane, 1-heptanol, 2-propen-1-ol, decamethyl-cyclopentasiloxane, α-Thujene, β-pinene, 2-ethylhexanal, 2-propanone, pentanal, and pentanol.

These VCs were not detected in the raw CMA nuggets with the incorporation of PF, except α-Terpinene, γ-Terpinene, hexanal and terpinene-4-ol, which were detected only in the control samples (without PF). On the other side, in comparison with the raw samples, the contents of these VCs increased after cooking by different methods; however, most of them did not appear in the samples treated with 6 and 12% of PF. In general, deep-frying and microwave resulted in the higher levels (darker orange color) of these VCs compared to the other cooking methods.

The increase of these volatile might be related to heat treatments. Zhang et al. (2023) reported that terpenes, aldehydes, ketones, and esters, are mostly generated by degradation and oxidation. Moreover, the formation of some aldehydes including hexanal, pentanal and 2-ethyl hexanal and alcohols is due to the oxidation of lipids induced by heat treatment (Sohail et al., 2022). Hexanal and pentanal are recognized for their role in the rancid flavors, which is the main indicator of high fat food degradation (Qi, Xu, 2021). The formation of 2-propanone may be due the β-keto acid decarboxylation or the β-oxidation of saturated fatty acids (Shi et al., 2019).

The second cluster comprised of isoamyl acetoacetate, (E)-β-Ocimene, (Z)-β-Ocimene, ethyl 2-hexenoate, 3-ethyl-pentan-2-ol, 3-methyl-4-octanone,3-ethyl pentane, limonene, and myrcene. The content of these VCs increased significantly with the increase of PF concentration in the raw samples. After cooking, their concentrations, in general, tended to be stable being these compounds the majority group in all samples.

The third cluster included esters and alcohols such as ethyl isovalerate, methyl hexanoate, 1-hexanol, 3-methylbutanoic acid 3-methylbutyl ester, 3-methyl-1-butanol, 2-methyl-1-butanol, amyl formate, nonyl aldehyde, ethyl 2-methylbutyrate, isopropyl 3-methylbutanoate, and isobutyl isovalerate. Most of them were decreased in the raw samples with the incorporation of PF, while others increased, especially ethyl 2-methylbutyrate, isopropyl 3-methylbutanoate, isobutyl isovalerate. However, all of these esters and alcohols were not identified in the cooked samples as compared to raw. The absence of them in the cooked samples suggests that thermal treatments degraded these VCs. Finally, the fourth cluster comprised of hexane, ethyl butanoate, 2-β-pinene, m-cymene, β-elemene, sabinene and cis-sabinene hydrate acetate. All of these VCs were perceptively decreased with the addition of PF in raw samples. On the other hand, these VCs did not appear after cooking. This might be as mentioned above, due to thermal treatments.

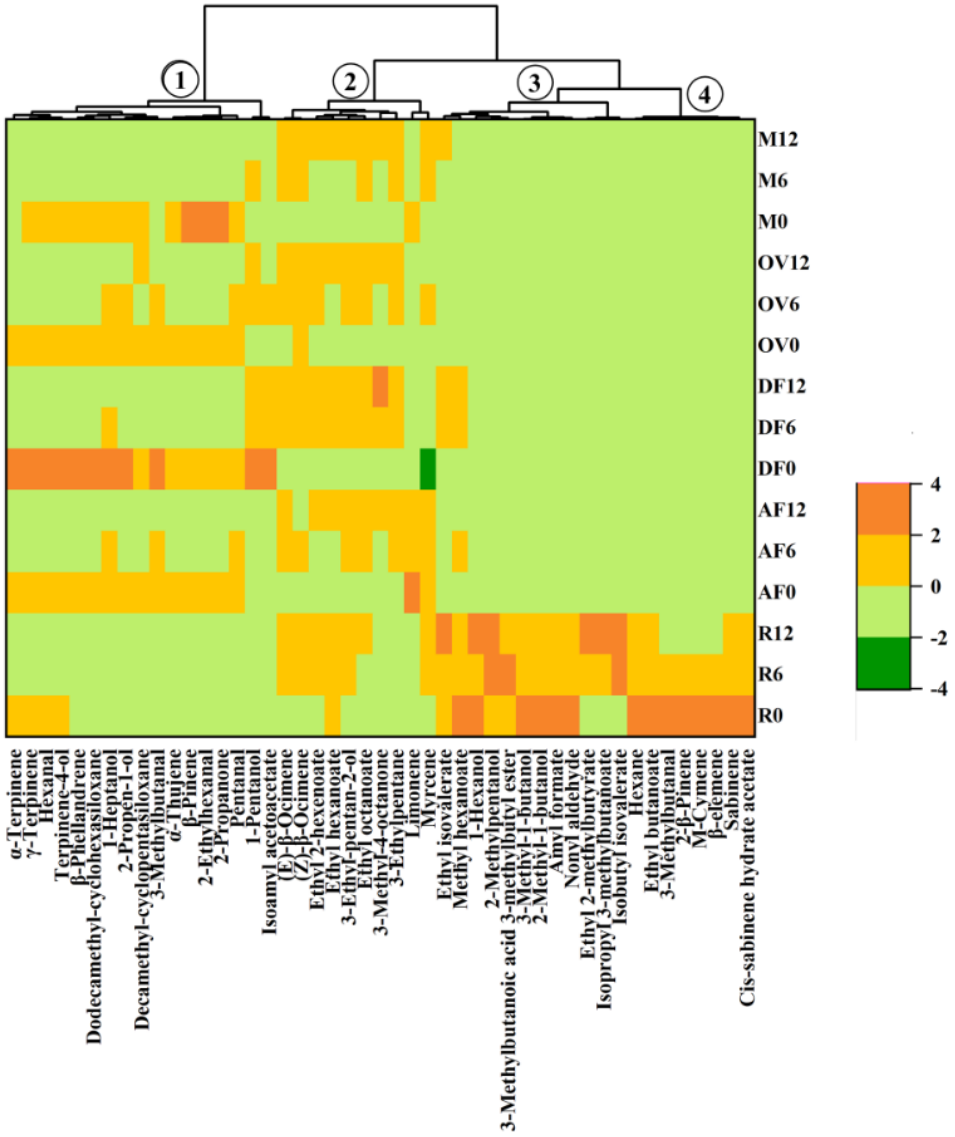

Fig. 2. Heat map of different volatile compounds of chickpea meat analogue (CMA) nuggets treated with different cooking methods.

3.4.2. Principal component analysis (PCA)

In order to highlight the differences in VCs in different raw and cooked CMA nuggets by different cooking methods, PCA was performed. The first two PC1 and PC2 explained 74.94% of the total variance (44.99% and 29.95%, respectively). PC1 was the most important variable to explain the differences between the treatments. According to loading values (Table 3), α-thujene, β-phellandrene, γ-terpinene, 1-heptanol, 2-propanone, 2-propen-1-ol, β-pinene, 3-methylbutanal, dodecamethyl-cyclohexasiloxane, decamethyl-cyclopentasiloxane, hexanal, 2-ethylhexanal, pentanal, terpinene-4-ol were negatively correlated with PC1, whereas (E)-β-ocimene, (Z)-β-Ocimene, ethyl isovalerate, ethyl hexanoate, methyl hexanoate, amyl formate, 1-hexanol, 2-methylpentanol, and 3-methylbutanoic acid 3-methylbutyl ester were positively correlated with the same dimension.

On the other side, PC2 was negatively correlated with (E)-β-ocimene, ethyl 2-hexenoate, 3-ethyl-pentan-2-ol, ethyl octanoate and 3-ethylpentane while γ-terpinene, hexanal, terpinene-4-ol, hexane, 3-methylbutanal, 3-methyl-1-butanol, 2-methyl-1-butanol, amyl formate, ethyl butanoate, 1-hexanol, sabinene, 2-β-pinene, m-cymene, cis-sabinene hydrate acetate, nonyl aldehyde, 3-methylbutanoic acid 3-methylbutyl ester and β-elemene were positively associated with the PC2. The score plot (Fig. 3B) indicates that the samples were divided in three groups. Group 1, wich is situated in the first quadrant (Q1), is composed of the raw samples (R0, R6, R12). This group was correlated with most VCs (20 compounds). Group 2, located in the second quadrant (Q2), is formed by cooked samples with 6% and 12% of PF (AF6, AF12, DF6, DF12, OV6, OV12, M6 and M12). This group was characterized by 9 VCs. Group 3, located in the third quadrant (Q4), formed by cooked samples without pequi (AF0, DF0, OV0 and M0) was characterized 16 VCs.

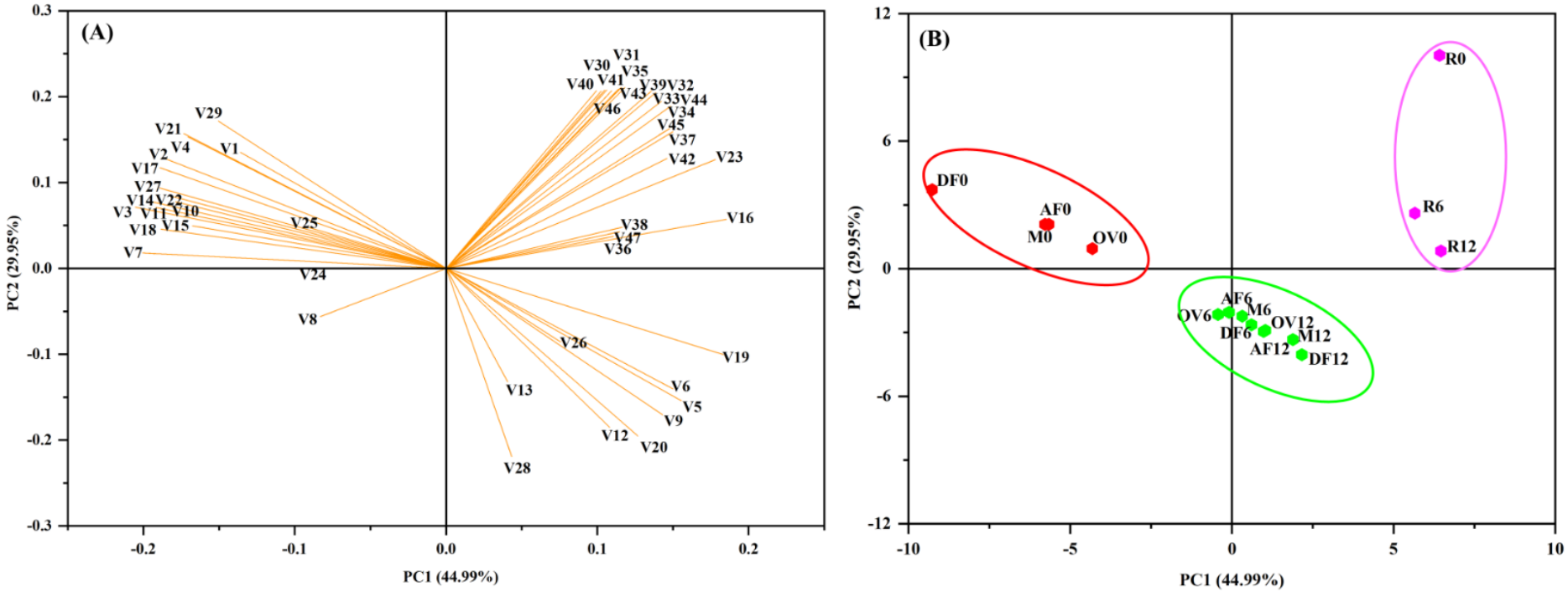

Fig. 3. Principal component analysis (PCA) of the volatile compounds of chickpea meat analogue (CMA) nuggets. (A) Scores bi-plot (B) Loadings bi-plot. R: raw CMA nuggets; AF, air-fried CMA nuggets; DF, deep-oil fried CMA nuggets; OV, oven-roasted CMA nuggets; M, microwaved CMA nuggets; 0: 0% of PF; 6: 6% of PF; 12: 12% of PF; V1, α-Terpinene; V2, α-Thujene; V3, β-Phellandrene; V4, γ-terpinene; V5, (E)-β-ocimene; V6, (Z)-β-ocimene; V7, 1-heptanol; V8, 1-pentanol; V9, ethyl 2-hexenoate; V10, 2-propanone; V11, 2-propen-1-ol; V12, 3-ethyl-pentan-2-ol; V13, 3-methyl-4-octanone; V14, β-pinene; V15, 3-methylbutanal; V16, ethyl isovalerate; V17, dodecamethyl-cyclohexasiloxane; V18, decamethyl-cyclopentasiloxane; V19, ethyl hexanoate; V20, ethyl octanoate; V21, hexanal; V22, 2-ethylhexanal; V23, methyl hexanoate; V24, isoamylacetoacetate;V25, limonene; V26, myrcene; V27, pentanal; V28, 3-ethylpentane; V29, terpinene-4-ol; V30, hexane; V31, 3methylbutanal; V32, 3-methyl-1-butanol; V33, 2-methyl-1-butanol;V34, amyl formate; V35, ethyl butanoate; V36, ethyl-2-methylbutyrate; V37, 1-hexanol; V38, isobutyl isovalerate; V39, sabinene; V40, 2-β-pinene; V41, m-cymene; V42, 2-methylpentanol; V43, cis-sabinene hydrate acetate; V44, nonyl aldehyde; V45, 3-methylbutanoic acid 3-methylbutyl ester; V46, β-elemene; V47, isopropyl 3-methylbutanoate.

4. Sensory analysis

The effects of the four cooking methods (air-frying, deep-oil frying, oven-roasting and microwave) on sensory attributes of CMA nuggets (control samples without PF) is given Fig.4. The main parameters affecting eating quality attributes, such as appearance, aroma, taste, texture and overall acceptance were measured. Overall, all the attributes were significantly ($p < 0.05$) affected by the cooking methods, except aroma which showed little differences but without practical significance. Deep-oil fried CMA nuggets received the significant ($p < 0.05$) highest appearance, taste and overall acceptance scores, followed by air-fried and oven-roasting samples, whereas the lowest scores for these attributes were recorded in the microwaved samples.

In terms of texture, air-fried, deep-oil fried and oven-roasted samples revealed the highest ($p < 0.05$) scores without statistical differences among them ($p > 0.05$), while microwaved samples showed the lowest scores. The lowest overall acceptance score of the microwaved samples might be related to the higher L* and lower a* and b* (data not shown), which greatly contributed to the appearance and color of theses samples. Moreover, the low crispy texture and low hardness of the microwaved samples (data not shown) might also have contributed to the lowest overall acceptance.

On the other hand, deep-oil fried CMA nuggets showed higher appearance due to the golden color formed on the surface resulted from the Maillard reaction and caramelization of carbohydrates from the ingredients used in coating process during deep-frying. In addition, the oil used might have increased the fat amount of the samples resulting in more attractive flavor and texture attributes that enhance the mouthfeel sensation of the consumers (Kupirovič et al., 2017). Therefore, deep-oil frying was the more acceptable method for cooking CMA nuggets.

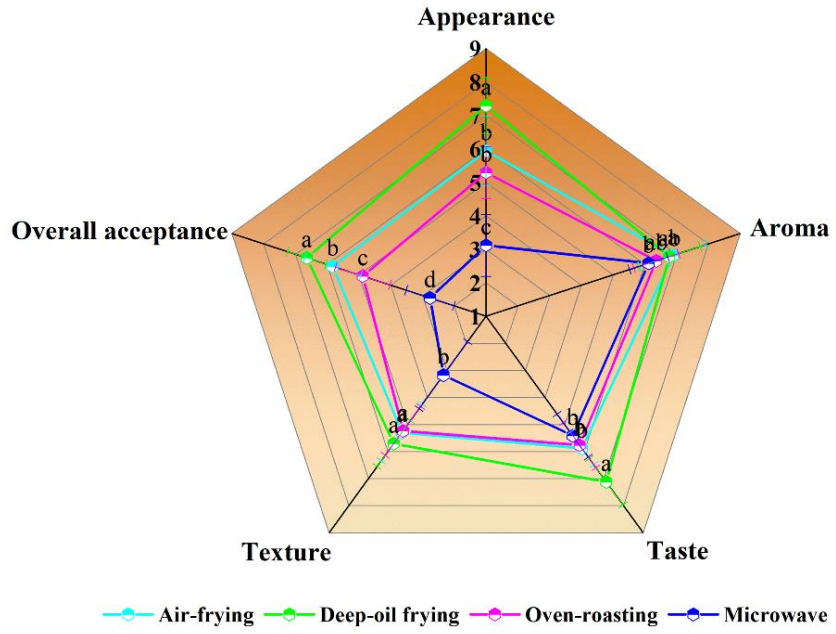

Fig.4. Effect of cooking methods on the sensory attributes of chickpea meat analogue (CMA) nuggets. Different letters (a-d) indicate significant differences between different cooking methods for each sensory attribute ($p < 0.05$) by Tukey test.

4. Conclusions

In general, this study demonstrated that incorporation of pequi flour and cooking methods had significant impact on the quality of CMA nuggets. Addition of pequi flour increased the contents of carotenoids, monounsaturated fatty acids (MUFA), saturated fatty acids (SFA), terpenes, and esters and decreased the cooking loss and the contents of polyunsaturated fatty acids (PUFA). Deep-oil fried CMA nuggets resulted in better fatty acid profile, volatile compounds and presented better sensory characteristics, therefore, was considered to be the suitable cooking method for CMA nuggets.

Acknowledgments

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