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The antimicrobial, antioxidant and sensory properties of garlic and its derivatives in Brazilian low-sodium frankfurters along shelf-life

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ABSTRACT

The goal of this study was to evaluate the effect of garlic extract obtained by pressurized liquid extraction (PLE) when compared to conventional garlic products (fresh, powder, commercial oil) added in low cost and low sodium frankfurters (50% NaCl substituted by KCl and $CaCl_2$) on the physicochemical and microbiological properties during shelf-life. The highest allicin content was detected in the PLE extract, followed by fresh garlic and garlic powder. Fresh garlic behaved as potential antioxidants and antimicrobials against spoilage bacteria during shelf life. The overall acceptability of the frankfurters containing commercial garlic oil (F3) showed the worst score when compared to the control formulation. The use of the PLE extract has shown to be an antioxidant on typical reduced sodium emulsified meat products formulations.

1. Introduction

Sodium reduction in meat products is a mandatory goal to meet governmental regulatory recommendations aimed at promoting healthier claims and reducing the risk of many chronic diseases, such as hypertension, cardiovascular and renal diseases, and some types of cancer (WHO, 2012). Because NaCl is the main source of sodium in meat products, this compound has been targeted for reduction in industrial formulations. Salt reduction is challenging given the multiple functions of this ingredient, such as conferring suitable texture (due to the increase in ionic strength and extraction of myofibrillar proteins) and flavor, besides its food preservation properties (Desmond, 2006).

Typical Brazilian frankfurters represent one of the most consumed meat products, which may contain up to 60% of mechanically deboned poultry meat (MDPM), as permitted by the current legislation (Brasil, 2000). Because MDPM contains high collagen and heme pigment content and low myofibrillar protein levels, sodium reduction must be applied together with additional technological barriers to guarantee product safety and stability (Day & Brown, 2001). The addition of effective natural products with antioxidant and antimicrobial properties, such as garlic and its derivatives, which are already part of the formulation as condiments, can provide substantial benefits to the consumers of frankfurters.

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The properties of garlic and its components have been intensely studied due to their important physiological, therapeutic and medicinal properties (Santhosha, Jamuna, & Prabhavathi, 2013), including antioxidant action against free radicals, immune system stimulation, cardiovascular disease prevention, and anticancer actions (Colic & Savic, 2000; Herman-Antosiewicz, Powolny, & Singh, 2007; Khanum, Anilakumar, & Viswanathan, 2004).

From a technological standpoint, the use of fresh garlic and its derivatives has been proposed by many authors due to their antioxidant and antimicrobial (antibacterial, antifungal, and antiparasitic) activity, especially against the development of pathogens (Haciseferogullari, Oscan, Demir, & Calisir, 2005; Yin & Cheng, 2003). The rupture of garlic bulbs leads to the formation of thiosulfinates, in which the precursor compound alliin is transformed into allicin (diallyl thiosulfinate) by enzymatic action; allicin is the most important bioactive compound in garlic due to its broad functional activity and high concentrations (Lawson & Hughes, 1992). However, alliinase, which catalyzes the conversion of alliin to allicin, is thermolabile, as is allicin; the latter decomposes within a few hours to form more stable sulfur compounds that have reduced activity (Rybak, Calvey, & Harnly, 2004).

In this context, new extraction processes from natural matrices while maintaining the bioactive compounds have been developed, such as pressurized liquid extraction (PLE) for being a rapid (<30 minutes) and low-cost technique, and presenting lower levels of solvent and low environmental impact as technological advantages. In addition, this process associated with ethanol (GRAS and economical)

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can facilitate the extraction of compounds of interest, including organosulfur and phenolic compounds, once the extraction in a closed system can preserve various light-sensitive compounds and oxygen. Finally, the extraction efficiency targeting higher performance and preservation of bioactive compounds may be possible by setting parameters such as temperature (40–139 °C) and pressure (3.5 to 20 MPa) (Mustafa & Turner, 2011; Osorio-Tobón & Meireles, 2013).

Hence, the present study aimed to evaluate the effect of the extract obtained by pressurized liquid extraction when compared to different garlic products (fresh, powder, commercial garlic oil) as antioxidant and antimicrobial compounds in frankfurters with high MDPM content and 50% NaCl replaced by blends of salt substitutes ($CaCl_2$ and KCl) throughout its shelf life.

2. Materials and methods

2.1. Garlic derivatives

Fresh garlic and its derivatives were prepared for use in the frankfurters, as follows: a) Fresh garlic (*Allium sativum* L., family: Liliaceae): Garlic was purchased in a food market in Campinas. The garlic was manually peeled in a refrigerated environment and grounded in a blender (Retsch, Germany) immediately before the addition to the food mixture to prevent the degradation of the sulfur compounds. b) Garlic powder: A commercial "pure garlic powder" product was used, which is manufactured by a renowned company in the spice sector and commonly used in the industrial preparation of frankfurters (Fuchs Gewürze, Brazil). The chloride level was determined to guarantee absence of NaCl and to adjust the moisture content to levels equivalent to that of fresh garlic. The time between production and use in frankfurter processing: no more than 2 months. c) Commercial garlic oil: This product was donated by a traditional food ingredient company (IFF International Flavors & Fragrance Inc.). The time between production and use in frankfurter processing: no more than 3 months. d) Garlic extract obtained by pressurized liquid extraction (PLE extract) according to Farías-Campomanes, Horita, Pollonio, and Meireles (2014). The garlic extract was obtained at the Supercritical Laboratory of Technology (LASEFI/DEA/UNICAMP, BRAZIL) using pressurized liquid (ethanol) extraction on a bed containing fresh garlic prepared according to item a. The garlic PLE extract was obtained at 40 °C and 6 MPa, using ethanol as a solvent due to it is generally recognized as safe (GRAS), besides its chemical affinity for the desired extraction compounds. The PLE extract was obtained the day before meat processing and stored at −18 °C until use (no more than 1 month).

2.2. Experimental design

In accordance with previous studies (Horita, Messias, Morgano, Hayakawa, & Pollonio, 2014; Horita, Morgano, Celeghini, & Pollonio, 2011), a salt blend containing 25% KCl, 25% $CaCl_2$, and 50% NaCl was selected as a substitute for 50% NaCl in all frankfurter formulations, except for C2, which had only lower NaCl level. The percentages were calculated to obtain an ionic strength equivalent to 2% NaCl, resulting in 1.00% NaCl + 0.31% $CaCl_2$ + 0.63% KCl. The levels of garlic and its derivatives and the respective treatments are presented in Table 1 and are based on previous studies on the limiting saturation of garlic flavor tolerable to trained assessors (1% for fresh garlic). The garlic powder content (0.3%) was equivalent to the fresh garlic levels, considering the moisture content experimentally determined in the ingredient. The level of the commercial garlic oil was 0.006%, following the recommendations of the manufacturer, which was determined in preliminary tests. Because PLE extract is a new compound little studied in literature, a percentage of 0.2% was used based on its enhancer property investigated in preliminary tests.

Table 1
Levels of garlic derivatives (%w/w) used in the formulation of low-salt frankfurters.

Treatments	Fresh garlic[a]	Powder garlic[b]	Commercial garlic oil[c]	Garlic PLE extract[d]
C1	0	0	0	0
C2	0	0	0	0
F1	1.0%	0	0	0
F2	0	0.3%	0	0
F3	0	0	0.006%	0
F4	0	0	0	0.2%

C1 — Control (2.00% de NaCl without garlic derivatives).
C2 — Blends Control (1.00% NaCl + 0.31% $CaCl_2$ + 0.63% KCl without garlic derivatives).
F1, F2, F3 e F4 — Blends of chloride salts (1.00% NaCl + 0.31% $CaCl_2$ + 0.63% KCl) with garlic derivatives.
PLE — pressurized liquid extraction.
[a] Obtained of specialized local trade.
[b] Fuchs Gewürze, Brasil.
[c] IFF International Flavors & Fragrance Inc.
[d] Lasefi, FEA, Unicamp.

2.3. Formulations and processing

The fresh garlic and its derivatives were removed from treatments C1 and C2, which constituted the control formulations. All formulations contained poultry MDPM (600 g/kg), pork meat (140 g/kg), pork back fat (100 g/kg), ice (100 g/kg), cassava starch (20 g/kg), isolated soy protein (10 g/Kg), spices in powder (white pepper, onion and paprika — 4 g/Kg), sodium tripolyphosphate (3 g/kg), sodium erythorbate (4 g/kg), and sodium nitrite (0.15 g/kg). Both the salt content at ionic strength equivalent to 2% NaCl (FC1) and the amount of raw materials were kept constant, and the difference to result in 1000 g of meat batter was discounted from the water content. Refrigerated pork meat (−1 to −2 °C) was ground using 5-mm-orifice discs, and the pork back fat was ground using 3-mm-orifice discs. The emulsion was processed in a cutter (Mado®): MDPM, pork meat, salt, half the amount of water, sodium nitrite, sodium tripolyphosphate, condiments and garlic and its derivative were mixed, and the ingredients were ground until the temperature reached 7 °C. Next, the remaining water was added along with sodium erythorbate and pork back fat. Then, cassava starch and soy protein isolate were added, and the comminution process was continued while ensuring that the maximum batter temperature did not exceed 12 °C. The batter was stuffed into permeable artificial cellulose casings (10 mm) in a stuffer (Mainca®) and cooked in an oven (ARPROTEC®, Brazil) according to the following cooking program: 15 minutes at 65 °C and 98% relative humidity (RH), 20 minutes at 75 °C and 98% RH, and then 85 °C and 98% RH until the internal temperature reached 72 °C. Then, the products were cooled down in cold drinking water and stored in a cold chamber until the product temperature reached 5 °C. The casing of each frankfurter was removed, and the external surface of the frankfurters was dyed in annatto solution and the frankfurters were immersed in citric acid solution to fix the color, followed by washing in water to eliminate the excess acid and dye. Finally, the frankfurters were vacuum packaged nylon/polyethylene bags (9.3 ml $O_2/m^2/24$ h) and stored in a cold chamber at 5 °C for 60 days.

2.4. Physicochemical analysis

2.4.1. Determination of allicin content

The garlic samples were prepared for determination of the allicin content according to the internal standard (IS) method by Mendes (2008). The quantification of allicin was performed in a high-performance liquid chromatography (HPLC) system (Waters Alliance separation module, 2695D, Milford, USA) equipped with a diode array detector (DAD). The identification of allicin in the garlic and its derivatives was performed according to the methodology described by del