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Quality of frankfurter-type sausage with added pork liver as source of retinol and minerals

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Abstract

Micronutrient deficiencies in developing countries are due to an inadequate diet, an incomplete diet, or low nutrient availability. The prevalent deficiencies are of vitamin A, iron, zinc, and selenium, which occur mainly during old age, pregnancy, and childhood. Foods rich in bioactive compounds, such as pork liver, can be used as ingredients to reformulate traditional meat products. The present work focused on developing a frankfurter-type sausage with pork liver (PL) as a retinol, iron, zinc, and selenium source, and evaluating the sausage's quality during cold storage. The PL was added at three different concentrations: control (without PL addition), T1 (4% PL), T2 (8% PL), and T3 (12% PL). When the PL in the meat formulation increased, the iron, zinc, selenium, and retinol contents also increased to 20.0 mg/100 g, 11.1 mg/100 g, 130.4 µg/100 g, and 1,188 µg/100 g, respectively. However, lipid oxidation also increased as the PL increased after 60 d of cold storage (0.29, 0.50, 0.61, and 0.74 mg MDA/kg in the control, T1, T2, and T3 treatments, respectively). Adding PL did not affect the fatty acid profile of the products. Additionally, adding PL did not affect the texture profile, but cold storage increased all of the parameters, especially in high pork liver formulations. The addition of PL decreased the *L** values, and increased the *a** values. The frankfurter-type sausage developed in the present work could be a food with a good retinol content, and a dietary source of minerals such as iron, zinc, and selenium. It could also be used as a food option for sectors of the population with special needs.

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Introduction

Vitamin, mineral, and trace element deficiencies persist in different populations worldwide (WHO, 2023), mostly in regions of Africa, Asia, and Latin America (Stevens *et al.*, 2015; Gupta *et al.*, 2020). Some highly prevalent deficiencies include vitamin A and minerals such as iron, zinc, and selenium, which are present mainly in older adults, pregnant women, and children. Older adults are prone to develop vitamin and trace element deficiencies due to decreased quantitative and qualitative food intake, loss of metabolically active body cell mass, and the development of chronic age-associated disorders (Hoffman, 2017). Ramírez-Silva *et al.* (2020) reported a prevalence of 48.2 and 60.7% zinc and vitamin A deficiency, respectively, in the Mexican adult population. In pregnant women, micronutrient status can become compromised due to an increase in the need for vitamin A, folic acid, iron, and zinc (Gernand *et al.*, 2016). The estimated prevalence of prenatal iron deficiency worldwide is 15 - 20%, whereas vitamin A deficiency affects an estimated 15% of pregnant women in low-income countries (Bailey *et al.*, 2015). In relation to children, one-third of the population in low- and middle-income families has vitamin A deficiency (UNICEF, 2023). In Mexico, more than 25% of children under 5 years of age are vitamin A deficient (IMSS, 2014). A WHO report estimated that deficiencies in vitamins and minerals, particularly iron, vitamin A, and zinc, affect 50% of preschool-aged children and 67% of women of reproductive age worldwide (WHO, 2023). These micronutrient deficiencies are associated with a

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greater risk of blindness, a weakened immune system, and a reduced physical capacity (Pecora *et al.*, 2020). Additionally, mothers with low levels of micronutrients may have babies who are born prematurely or with low birth weights (Jamshed *et al.*, 2020; Boskabadi *et al.*, 2021). According to WHO, there are 10.8 million child deaths globally every year, and the number attributed to iron, zinc, and vitamin A deficiencies accounts for approximately 19% of the total.

Nutritional deficiencies can be mitigated with a varied, balanced, and adequate diet that provides sufficient amounts of micronutrients to meet the daily requirements of different population groups. However, these daily requirements are sometimes difficult to achieve due to the lack of access to all food groups or high requirements at certain stages of life; for example, children do not need micronutrients in the same amounts as adults (Maggini *et al.*, 2018). According to Bailey *et al.* (2015), the prevalent deficiencies in micronutrients in developing countries are due to inadequate food intake, poor dietary quality, and low bioavailability of nutrients. More research is needed to increase the food supply that meets the micronutrient needs of the population, and a good strategy is food fortification, especially for those that are commonly consumed, such as meat products.

Research on the development of food products that positively affect the health of all populations has increased in recent years (Topolska *et al.*, 2021). According to Gabdukaeva *et al.* (2021), the meat product market is developing rapidly due to the increased demand for ready-to-eat products. In addition, offering meat products with a balanced composition can improve the nutritional status and health of children, pregnant women, and elderly individuals with special nutritional needs.

More people are interested in healthier meat products with an accessible cost and that are sensorially acceptable. In this sense, pork has a significant nutritional contribution to the diet due to its high-quality protein content, B complex vitamins, and minerals such as iron, zinc, and selenium (Haytowitz *et al.*, 2019). In Mexico, pork is cheaper than beef or chicken, and its per capita consumption is 19 kg, ranking eighth worldwide. The Mexican Meat Council (COMECARNE, 2022) revealed that the consumption of pork has increased in the last decade, and the product made from it with the highest production and consumption was the frankfurter. Meat product consumption in Mexico increased by 6.6% from 2020 to 2021, with frankfurter being the most popular, accounting for 66% from the total (COMECARNE, 2022).

During pig slaughter, by-products with high nutrient contents are generated, one of which is the liver, which is characterised by high vitamin A, iron, zinc, and selenium contents (Seong *et al.*, 2014). In Mexico, the liver is consumed as a traditional dish, and usually cooked and seasoned with onion, garlic, pepper, and salt. Pork liver destined for human consumption in Mexico is obtained from Federal Inspection Type Slaughterhouses, facilities subject to regulation by the Ministry of Agriculture, Livestock, Rural Development, Fisheries, and Food. Owing to its nutritional profile and traditional consumption, pork liver has the potential to be used as an ingredient in the formulations of special meat products such as frankfurters. The addition of non-traditional ingredients into a meat product formulation could affect the quality of the product. Therefore, in the first stage, within a series of stages necessary to achieve a functional meat product, the present work focused on the formulation of a frankfurter-type sausage with pork liver as a source of retinol, iron, zinc, and selenium, and then evaluated its quality parameters during refrigerated storage.

Materials and methods

Raw materials and additives

Fresh pork semimembranosus muscle and liver samples were obtained from a Federal Inspection Type Slaughterhouse (TIF slaughter facility) in Hermosillo, Mexico, after 24 h post-mortem (2 - 4°C in refrigeration). Pork meat and liver were used immediately after they were obtained from the slaughterhouse. The ingredients used for the pork frankfurters were salt (Salt Bay®, Mexico), sodium polyphosphate (Piasa®, Apodaca, N.L., Mexico), curing salt (6.25% nitrite; Fabpsa®, Ciudad de Mexico, Mexico), sodium erythorbate (Fabpsa®, Ciudad de Mexico, Mexico), frankfurter seasoning (Excalibur Seasoning Company, Ltd., Pekin, IL, USA), and chickpea (Abarrey®, Sonora, Mexico). The chickpea was cooked in boiling water for 1 h, and used as a paste in the meat formulation (the moisture, protein, fat, and ash contents of the cooked chickpea were 62.41, 7.22, 0.41, and 2.72%, respectively).

Manufacturing of frankfurter-type sausages

The excess fat and visible connective tissue were trimmed from lean pork or raw pork liver, cut into small pieces, and chopped with salt, polyphosphates, curing salt, sodium erythorbate, and half of the ice for 2 min using a bowl cutter (Kilia Fleischereimaschinenfabrik, Kiel, Germany). Ice water, chickpea, and seasoning were then added, and the mixture was blended under vacuum for an additional 3 min. During the entire emulsification procedure, the batter temperature was not higher than 10 ± 2°C. The final batter was stuffed into cellulose casings (20 mm diameter) using automatic equipment (Omet ICS60-B, Siena, Poggibonsi, Siena, Italy). The samples were hand-linked and cooked in an EnviroPak oven (CVU350E, Clackamas, OR, USA) to an internal temperature of 71.1°C, which was monitored *via* a thermocouple inserted in the centre of a frankfurter. The frankfurters were subsequently cooled in ice water (2°C) for 10 min, vacuum packed (Supervac GR-185, Vienna, Austria), and stored at 2°C for further analysis. Two replicates of the experiment were performed on different days. Four different formulations of frankfurter-type sausage (approximately 5 kg, 100 frankfurter pieces) were manufactured in each replicate as follows: a control without pork liver added, and three formulations with the addition of 4% (T1), 8% (T2), and 12% (T3) pork liver. These formulations ensured a significant contribution of retinol and minerals to the sausage, and were chosen to detect potential losses that may occur due to degradation during processing and refrigerated storage. These findings would provide a basis for additional research on the bioaccessibility and bioavailability of these components. This would ultimately enable adjustments to be made to the formulation, ensuring that it contains adequate and sufficient quantities in line with daily nutritional requirements.

The pork frankfurters were then stored at 2°C for 60 d. Quality parameter evaluations were conducted at least in triplicate for each independent replicate. The proximate composition, mineral contents (iron, zinc, and selenium), and fatty acid profile were determined only on day 0. Retinol content, instrumental colour, pH, texture profile analysis, and TBARS were conducted on days 0, 12, 36, and 60 to monitor the effects of storage on quality characteristics.

Proximate composition

Standard methods proposed by the Association of Official Analytical Chemists (AOAC, 2016) were used for analyses of moisture (950.46), crude fat (960.39), protein (920.53), and ash (938.08).

Iron, zinc, and selenium contents

The contents of iron, zinc, and selenium were determined in triplicate following the AOAC method 968.08 (AOAC, 2016). In brief, 0.3 ± 0.003 g of sample previously lyophilised for 48 h (FreeZone 77530-00 Labconco Kingston, USA) was combined with 5 mL of concentrated nitric acid (Sigma Aldrich Co.), and digested for 4 h at 140°C on a heating plate. After the digestion process, the sample was diluted to 50 mL with HPLC water. Iron and zinc quantification was performed with an absorption atomic spectrophotometer (Varian, SpectrAA 240 FS) under an air-acetylene flame following the manufacturer's instruction. Before selenium quantification, 1 mL of concentrated nitric acid was added to 9 mL of previously digested samples, and heated in a water bath at 100°C for 45 min. The selenium concentration was measured with a 196 nm cathode lamp and hydride generation system (Varian Model VGA-77, Victoria, Australia). The certified reference material dogfish muscle DORM-4 from the National Research Council of Canada and blanks were used for quality control purposes. The resulting agreement between our data and the certified values was ≤ 10% for the three metals analysed.

Fatty acid profile

Fat was extracted from the pork frankfurters according to Bligh and Dyer (1959). Fatty acid derivatisation was carried out following the method reported by Park and Goins (1994). The resulting fatty acid methyl esters (FAME) were analysed *via* an Agilent gas chromatograph (Model 7890 B) equipped with an autosampler (Model 7693) and a flame ionisation detector (FID). Fatty acids were separated on a 100 m × 0.25 mm fused silica capillary column (SP-2560, Supelco, Bellefonte, PA, USA). The oven temperature was programmed from 150°C (20 min) to 220°C at a speed of 5°C/min. The injector temperature was set at 250°C, and the FID temperature was 300°C. Chromatograms were integrated into ChemStation software (ChemStation, Agilent Santa Clara, CA, USA). Fatty acids were

identified by comparing their retention times with those of commercial standards (Supelco 37 Component FAME Mix, Bellefonte, PA, USA). Fatty acids were expressed as a percentage of the total fatty acids detected. Saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and the PUFA/SFA ratio were then calculated.

Instrumental colour (CIE L, a*, b*)*

Instrumental colour was evaluated on each cooked frankfurter's surface and raw material (pork meat and liver) using a colorimeter (Chroma meter CR-400, Konica Minolta Sensing, Inc., Osaka, Japan). Five determinations were made per sample, and the average per treatment was reported. The colour measurement included the determination of the *L** (lightness), *a** (redness), and *b** (yellowness) values.

Texture profile analysis

Texture profile analysis was performed using a Texture Analyser TAXT2 (Stable Micro Systems, Ltd., Godalming, Surrey, UK). Frankfurter samples were cut into 2 cm cylinders, and compressed to 50% of their size twice *via* a 75 mm probe at a speed of 5 mm/s. The following texture profile attributes were calculated from the force-deformation curves (Bourne, 2002): hardness (N), springiness (cm), cohesiveness (adimensional), and chewiness (N/cm).

Thiobarbituric acid reactive substances in frankfurter-type sausages

Substances reactive to 2-thiobarbituric acid were quantified according to Rahman *et al.* (2019) with some modifications. Briefly, 5 g of the sample was homogenised (Ultra Turrax IKA Model T25) in 15 mL of 7.5% (w/v) trichloroacetic acid, EDTA, and propyl gallate (Sigma Aldrich) solution for 1 min. Subsequently, the homogenised sample was centrifuged at 2,500 *g* and 5°C for 15 min (Thermo Scientific Legend XTR refrigerated centrifuge, Pittsburgh, PA, USA). The supernatant was filtered through Whatman #1 paper; 2 mL of the filtrate was removed, and 2 mL of 0.02 M thiobarbituric acid (Sigma Aldrich) was added. Afterward, the sample was vortexed for 30 s (VX-200, Labnet International, Inc., NJ, USA), and heated in a water bath at 97°C for 20 min. The samples were cooled, and the absorbance was read at 532 nm using a UV-VIS spectrophotometer (Agilent Technologies, Cary 60 UV–VIS, Santa Clara, CA, USA). Lipid oxidation was calculated on the basis of the TBARS content against a standard curve of 1,1,3,3 tetramethoxypropane, and expressed as mg of malonaldehyde (MDA)/kg of product.

Retinol content

The retinol content was determined following the methodology reported by Hess *et al*. (1991), with some modifications. Briefly, 0.5 g of ground sample was weighed in 20 mL tubes, where 1 mL of 50% (w/v) NaOH (Sigma Aldrich) and 1 mL of absolute ethanol (Sigma Aldrich) were added. The samples were carefully mixed and placed in a water bath for 10 min at 80°C. The tubes were subsequently removed and vortexed for 10 s (VX-200, Labnet International, Inc., NJ, USA), and then returned to the water bath for an additional 20 min. The tubes were then allowed to cool in a cold-water bath. Next, 5 mL of hexane (Sigma Aldrich) was added, and the mixture was stirred for 30 s, and then centrifuged (Thermo Scientific Legend XTR refrigerated centrifuge, Pittsburgh, PA, USA) at 1,500 *g* and 10°C for 7 min. The tubes were allowed to stand until two layers formed. The hexane layer was separated and placed in a different tube, and dried under nitrogen flow; after that, 1 mL of ethanol (Sigma Aldrich) was added, and the mixture was poured into vials. Retinol was quantified *via* HPLC (Varian series 9010 Inc., CA, USA) with a UV-Visible 9050 detector and a C-18 column (10 cm × 4.6 mm ID, 3 µm) at a wavelength of 325 nm. The results were expressed as mg retinol per 100 g of sample.

Statistical analysis

Statistical analysis was performed using the Number Cruncher Statistical Systems 2020 (NCSS, Kaysville, UT) software. One-way analysis of variance (ANOVA) was performed to evaluate the effects of pork liver addition on quality characteristics (response variables on day 0: proximal composition, mineral content, and fatty acid profile), whereas Two-way ANOVA was applied to determine the effects of added pork liver and storage time (response variables: instrumental colour, texture profile analysis, TBARS value, and retinol content). Pork liver addition and storage time were assigned as fixed effects, and replication was assigned as a random effect. When a significant effect of the treatments was found

($p < 0.05$), the Tukey-Kramer test was performed. Mean differences were considered significant if the p value was lower than 0.05.

Results and discussion

Proximate composition

Table 1 shows the results of the proximate composition of the frankfurters with pork liver added. The moisture, fat, protein, and ash contents ranged from 68.6 to 70.3, 2.5 to 2.7, 16.6 to 17.8, and 2.0 to 2.5%, respectively. According to the technical regulation for sausage manufacturing (NMX-F-065-1984) issued by the Mexican government, these products have an upper limit of 70% for moisture, 30% for fat, and a lower limit of 9.5% for protein. A slight decrease in moisture content was observed in the formulations with the highest pork liver content, from 70.3% in the control treatment to 68.9 and 68.6% in the T2 and T3 treatments, respectively. This might have been due to the fact that most of the protein in pork liver is water-soluble protein, mainly albumin and groups of myofibrillar fragments (Steen *et al.*, 2016), with less water holding capacity. Studies reported by Nuckles *et al.* (1990) revealed that the water-soluble protein content was negatively correlated with reheat yield in cooked batters. On the other hand, the protein content did not significantly differ ($p > 0.05$) between the samples. In terms of composition, the frankfurters in the present work had moisture and protein values almost equal to those reported by Vanathi *et al.* (2020) for goat meat nuggets with 3.5% liver and kidney added. Compared with the control, T3 (12% pork liver) had a significantly greater ash content ($p < 0.05$). The increase in ash in the T3 treatment might have been due to the greater proportion of these components in the pork liver (2.9% ash) than in the pork meat (1.0% ash). Srebernich *et al.* (2015) also reported an increase in ash content from 1.7 to 3.4% when the percentage of pork liver increased from 0 to 13.3% in a meatloaf formulation.

Table 1. Proximate composition (g/100) of frankfurters formulated with different pork liver contents.

	Treatment				SEM
	Control	T1	T2	T3	
Moisture	70.3[a]	70.0[a]	68.9[b]	68.6[b]	0.71
Fat	2.6[a]	2.7[a]	2.5[a]	2.6[a]	0.23
Protein	17.5[a]	16.6[a]	17.7[a]	17.8[a]	0.31
Ash	2.1[a]	2.0[a]	2.2[a]	2.5[b]	0.24

Mean values in similar row with different lowercase superscripts are significantly different ($p < 0.05$). Control: 0% pork liver; T1: 4% pork liver; T2: 8% pork liver; and T3: 12% pork liver. SEM: standard error of the mean.

Mineral content

The mineral concentrations in the frankfurters with added pork liver are summarised in Figure 1. The contents of iron, zinc, and selenium ranged from 3.5 to 20.0 mg/100 g, 7.3 to 11.1 mg/100 g, and 68.0 to 130.4 µg/100 g, respectively. As the pork liver content increased from 0 to 12% in the frankfurter formulation, the contents of iron, zinc, and selenium also increased ($p < 0.05$). This might have been due to the high content of these components in the pork liver (76.5 mg/100 g, 14.7 mg/100 g, and 96.9 µg/100 g of iron, zinc, and selenium, respectively) compared with the pork meat (3.2 mg/100 g, 6.6 mg/100 g, and 64.9 µg/100 g of iron, zinc, and selenium, respectively). Srebernich *et al*. (2015) also reported an increase in mineral content when pork liver was added from 0 to 13.3% to a meatloaf formulation. They also reported increases in the iron and zinc contents from 1.65 to 4.17 mg/100 g and from 1.92 to 3.80 mg/100 g, respectively. Srebernich *et al*. (2017) evaluated the mineral level of a fortifying mixture containing pork liver prepared in powder form, and reported iron and zinc levels of 23.8 and 9.97 mg/100 g, respectively, which were almost equal to those of the T3 treatment in the present work (Figure 1). In Mexico, the average daily intake of heme iron, zinc, and selenium is 2.4 mg, 7.4 mg, and 37.6 µg, respectively (Tijerina-Sáenz *et al*., 2015; González-Torres *et al*., 2021). The prevalence reported for iron deficiency anaemia is 34.2% (Mejía-Rodríguez *et al*., 2021), for zinc deficiency is 33.8% (Gupta *et al*., 2020), and according to Liu *et al*. (2019), 80% of teenagers (10 to 18 years old) have a dietary intake of selenium below the recommended dietary allowance.

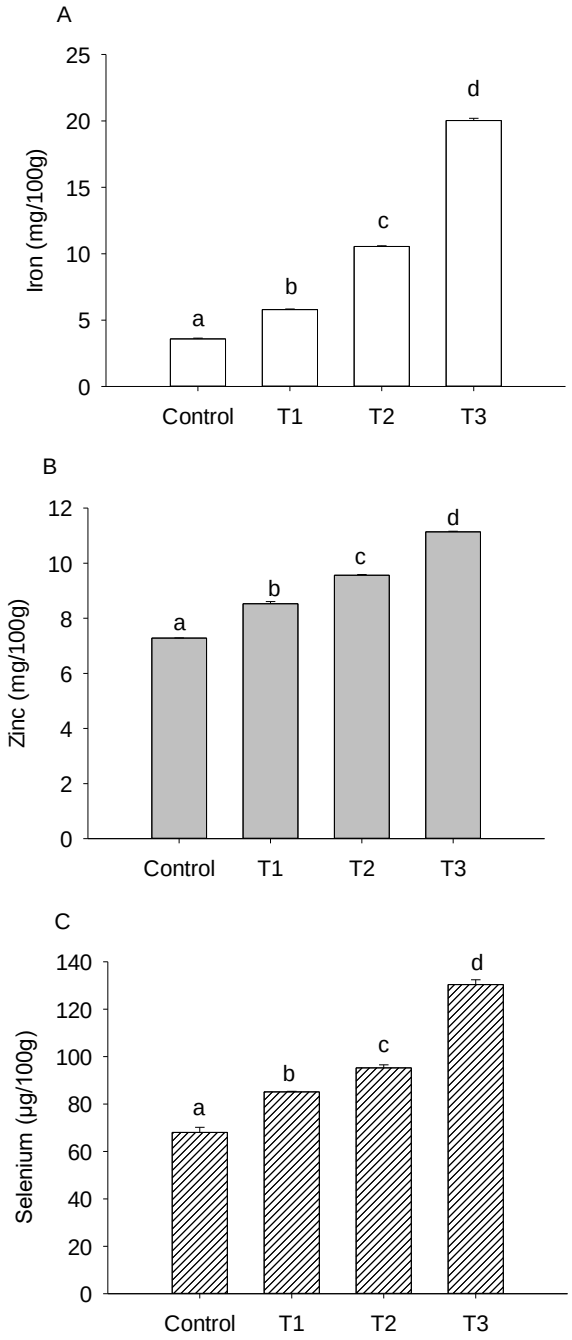

Figure 1. Effect of pork liver on mineral content of frankfurters. **(A)** Iron, **(B)** zinc, and **(C)** selenium. Error bars represent standard deviations. Bar charts with different lowercase letters within similar mineral group are significantly different ($p < 0.05$). Control: 0% pork liver; T1: 4% pork liver; T2: 8% pork liver; and T3: 12% pork liver.

To decrease the probability of deficiency, the recommended daily intakes for iron and zinc are 15 and 12 mg per day, respectively (Freeland-Graves *et al.*, 2020), and that for selenium is 55 µg per day for adults and children ≥ 14 years (Liu *et al.*, 2019); therefore, serving 100 g of the frankfurter-type sausage developed in the present work (T3 treatment) would ensure approximately 100% of these mineral requirements.

Fatty acid profile

The fatty acid profiles of the analysed products are presented in Table 2. There were no differences between treatments ($p > 0.05$) for any of the identified fatty acids. The fatty acids found in the highest proportion were monounsaturated (42.08 to 43.89%), followed by saturated (32.82 to 34.21%) and finally polyunsaturated (22.56 to 24.13%). The most predominant monounsaturated fatty acid was oleic acid (C18:1 *cis*), with a range of 38.17 to 40.1%. Among the saturated fatty acids, palmitic acid (C16:0) was found in the greatest proportion, followed by stearic acid (C18:0), which ranged from

Table 2. Fatty acid profile (g/100 g FAME) of frankfurters formulated with different pork liver contents.

	Control	**T1**	**T2**	**T3**	**SEM**
C14:0	1.2	1.2	1.2	1.1	0.01
C16:0	20.8	20.8	21.5	20.8	0.16
C17:0	0.4	0.4	0.4	0.2	0.05
C18:0	11.4	11.7	9.6	12.1	0.57
Σ SFA	33.8	34.1	32.8	34.2	1.25
C16:1	1.7	1.6	1.8	1.6	0.03
C17:1	0.2	0.2	0.3	0.2	0.06
C18:1*cis*	39.2	38.8	40.1	38.2	0.36
C20:1 n9	0.8	0.8	0.8	0.7	0.04
C22:1	0.2	1.4	0.9	2.5	0.46
Σ MUFA	42.1	42.8	43.9	43.2	0.78
C18:2 *cis*	19.7	19.5	19.8	18.7	0.22
C18:3 n3	1.1	1.1	1.2	1.1	0.03
C20:2	0.8	0.6	0.7	0.7	0.04
C20:3 n6	0.3	0.0	0.1	0.0	0.05
C20:3 n3	0.3	0.2	0.3	0.0	0.06
C20:4 n6	0.8	0.9	1.0	1.4	0.10
C20:5	1.2	0.7	0.1	0.7	0.26
Σ PUFA	24.1	23.1	23.3	22.6	0.67

Control: 0% pork liver; T1: 4% pork liver; T2: 8% pork liver; and T3: 12% pork liver. There were no significant differences among mean values in similar row ($p > 0.05$). SFA: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; FAME: fatty acid methyl ester; and SEM: standard error of the mean.

20.8 to 21.5% and 9.6 to 12.1%, respectively. The most abundant polyunsaturated fatty acid was linoleic acid (C18:2 *cis*), with values ranging between 18.7 and 19.8%. These results agreed with those of Freire *et al*. (2016), who reported similar values of fatty acid composition for frankfurters formulated with pork backfat.

Instrumental colour (CIE L, a*, b*) and texture profile analysis*

The colour parameters of frankfurter-type sausages affected by the addition of different pork liver contents during cold storage at 2°C are shown in Table 3.

Table 3. Changes in instrumental colour and texture of frankfurters formulated with different pork liver contents during storage (2°C for 60 d).

Parameter	Treatment	Storage day				SEM
		0	12	36	60	
*L**	Control	76.1^{aB}	74.6^{aA}	74.3^{aA}	74.0^{aA}	0.37
	T1	70.4^{bB}	69.1^{bA}	69.2^{bA}	69.1^{bA}	
	T2	66.2^{cB}	65.6^{cB}	65.1^{cAB}	63.9^{cA}	
	T3	62.1^{dB}	60.4^{dAB}	59.7^{dA}	59.1^{dA}	
*a**	Control	8.1^{aA}	7.5^{aA}	7.4^{aA}	7.7^{aA}	0.36
	T1	10.4^{bB}	10.1^{bB}	9.7^{abAB}	9.1^{abA}	
	T2	11.5^{bB}	10.1^{bAB}	10.7^{bAB}	9.7^{bcA}	
	T3	12.2^{bB}	12.1^{cB}	11.3^{cAB}	10.4^{cA}	
*b**	Control	17.2^{aA}	17.3^{aA}	16.8^{aA}	17.1^{aA}	0.18
	T1	17.4^{aA}	16.9^{aA}	16.8^{aA}	16.7^{abA}	
	T2	17.4^{aA}	17.0^{aA}	17.0^{aA}	17.2^{aA}	
	T3	16.3^{aA}	16.3^{aA}	16.3^{aA}	16.2^{bA}	
Hardness (N)	Control	54.5^{aA}	60.1^{aA}	66.1^{aB}	73.2^{aB}	1.29
	T1	56.3^{aA}	57.2^{aA}	67.6^{acB}	71.3^{aB}	
	T2	58.6^{aA}	63.5^{aA}	76.5^{bcB}	81.5^{bB}	
	T3	58.6^{aA}	61.9^{aA}	69.8^{abB}	79.6^{bB}	
Springiness (mm)	Control	0.87^{aA}	0.88^{aA}	0.89^{aA}	0.89^{aA}	0.01
	T1	0.86^{aA}	0.89^{aA}	0.89^{aA}	0.89^{aA}	
	T2	0.84^{aA}	0.87^{aAB}	0.88^{aB}	0.89^{aB}	
	T3	0.84^{aA}	0.87^{aAB}	0.88^{aB}	0.89^{aB}	
Cohesiveness	Control	0.72^{bA}	0.70^{bA}	0.70^{bA}	0.70^{aA}	0.01
	T1	0.70^{bA}	0.71^{bA}	0.70^{bA}	0.70^{aA}	
	T2	0.64^{aA}	0.66^{aA}	0.67^{aA}	0.68^{aA}	
	T3	0.62^{aA}	0.63^{aA}	0.66^{aA}	0.67^{aB}	
Chewiness (N/cm)	Control	34.0^{aA}	37.5^{bA}	42.1^{aB}	44.8^{aB}	1.15
	T1	34.0^{aA}	37.3^{abA}	44.8^{aB}	45.5^{aB}	
	T2	31.4^{aA}	37.3^{abA}	44.8^{aB}	44.5^{aB}	
	T3	31.2^{aA}	33.7^{aA}	42.4^{aB}	47.3^{aB}	

Control: 0% pork liver; T1: 4% pork liver; T2: 8% pork liver; and T3: 12% pork liver. Mean values in similar column with different lowercase superscripts are significantly different ($p < 0.05$). Mean values in similar row with different uppercase superscripts are significantly different ($p < 0.05$). SEM: standard error of the mean.

The lightness (*L**), redness (*a**), and yellowness (*b**) values ranged from 59.1 to 76.1, 7.4 to 12.2, and 16.2 to 17.7, respectively. The addition of pork liver to the frankfurter formulation had a significant effect ($p < 0.05$) on the *L** and *a** colour parameters of the products, but did not influence the

*b** value ($p > 0.05$) (Table 3). Based on the results of the present work, pork liver addition decreased lightness, and increased redness. These results were expected because the colour parameters of the product are related to the raw materials (Lorenzo *et al*., 2014). Compared with muscle, pork liver has a darker appearance, resulting in lower *L** values in the product. Pork liver also has high iron and heme iron contents (Seong *et al*., 2014), which influence the red colour of cured meat products. In these products, nitrites bind and temporarily stabilise the heme iron, forming a nitrosyl-myoglobin complex, and after cooking, the development of nitrosylhemochrome leads to the characteristic pink colour (Suman and Joseph, 2013). The results of the present work agreed with those of Rahman *et al*. (2019), who developed a reduced fat frankfurter by replacing beef tallow with defatted bovine heart, reporting a decrease in the *L** value, and an increase in the *a** value, with no effect on the *b** value as the content of defatted bovine heart increased in the frankfurter-type sausage formulation. Bovine heart, like pork liver, is an organ with high levels of total and heme iron (Valenzuela *et al*., 2009).

On the other hand, the storage time had a significant effect on the *L** and *a** values ($p < 0.05$), but not on the *b** values (Table 3). After 60 d of cold storage, the *L** and *a** values significantly decreased in all of the treatments evaluated, with less stability in the *a** value over time. The decrease in the *a** value was more evident in the T3 treatment, with high levels of pork liver added (12%). The T3 treatment resulted in a 15.2% decrease in the *a** value, whereas the control treatment resulted in only a 4.6% decrease after 60 d of storage. This significant decrease in the *a** value could have been due to the prooxidative activity of pork liver derived from its ability to release iron from heme pigments. As shown in Figure 2A, the T3 treatment with 12% pork liver had the highest TBA value compared with the other treated samples. A decrease in redness due to the oxidation process was also reported by Alirezalu *et al*. (2017) in sausages enriched with plant extract stored at 4°C.

Table 3 also shows the changes in the texture properties of frankfurter-type sausages containing pork liver during storage. The texture parameters of the frankfurter were affected by the addition of different pork liver contents and cold storage at 2°C for 60 d. The addition of pork liver to the frankfurter formulation had a significant effect ($p < 0.05$) on cohesiveness, but did not influence springiness or chewiness ($p > 0.05$). The samples with 8 and 12% pork liver had the lowest cohesiveness values ($p < 0.05$). Adding 8 and 12% liver to the formulation caused an increase in hardness ($p < 0.05$) from day 36 of storage. During the storage period, they caused an increase in hardness and chewiness ($p < 0.05$) in all formulations from day 36 of storage. The springiness of the 8 and 12% formulations also increased from day 36 ($p < 0.05$).

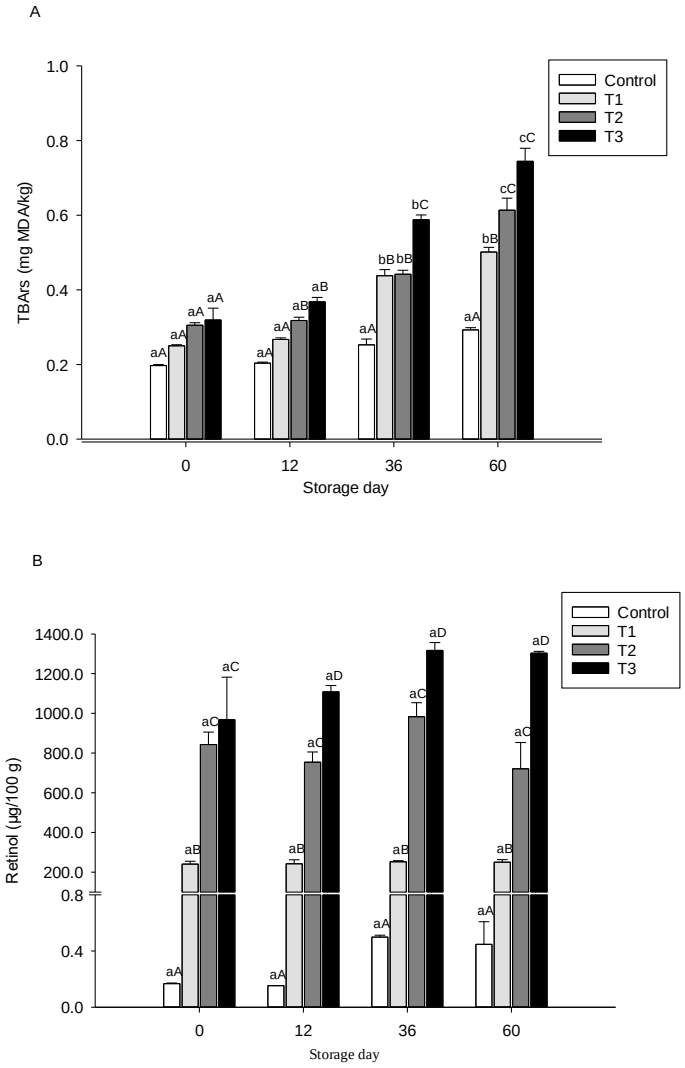

Figure 2. TBArs values **(A)** and retinol content **(B)** of frankfurters formulated with different pork liver contents during storage (2°C for 60 d). Error bars represent standard deviations. Bar charts with different letters show significant differences among the storage days (lowercase superscripts) in each treatment ($p < 0.05$) or among the treatments (uppercase superscripts) on each storage day ($p < 0.05$). Control: 0% pork liver; T1: 4% pork liver; T2: 8% pork liver; and T3: 12% pork liver.

In general, in all formulations, a gradual increase in hardness, springiness, cohesiveness, and chewiness was observed as the storage period increased. There is evidence of a positive relationship between the instrumental and sensory texture properties of meat products. Pematilleke *et al.* (2022) reported a strong correlation between instrumental and sensory texture properties in beef muscles. They concluded that texture profile analysis could effectively replace sensory analysis for measuring hardness and chewiness in meat products. Similar results were reported by Aguirre *et al.* (2018) for chicken breast fillets, who reported that sensory texture and instrumental texture profile analysis were accurate tools for measuring the texture of chicken meat. These findings are significant since the increase in texture properties observed in our study—particularly hardness and chewiness changes during storage—could negatively impact the sensory characteristics of our product. Although no sensory analysis was performed in the present work, instrumental texture measurements could provide insight into trends in sensory properties during cold storage. Importantly, compared with the T3 treatment with 12% pork liver, the T1 treatment with 4% pork liver resulted in a smaller increase in texture (Table 3); therefore, the impact on sensory properties would be expected to increase with increasing texture.

Some researchers have reported that cold storage of meat products could lead to increased instrumental texture and decreased sensory properties. Verma *et al.* (2022) reported that pork loaves containing 600 mg/g pork liver hydrolysate presented increased hardness and chewiness after 28 days of cold storage. Additionally, they noted a decline in the sensory properties of the product by the end of the storage period. Similarly, Amaral *et al.* (2015) added 25% liver into a lamb pâté formulation, and reported a significant increase in hardness along with a decrease in sensory texture after 90 days at 4°C.

The observed changes in texture might have been due to the loss of moisture that occurred in the frankfurters as the storage period increased. Changes in texture are more evident in meat formulations with the highest added pork liver (T2 and T3), possibly due to a decrease in myofibrillar protein from meat due to the substitution with pork liver. As discussed earlier, pork liver has a high water-soluble protein content with a low water-holding capacity (Steen *et al.*, 2016). Additionally, a negative correlation between water-soluble protein and yield in meat batter has been previously reported (Nuckles *et al.*, 1990). Several reports indicated that an increase in the hardness of the frankfurter was caused by a decrease in the moisture content of the product (Dharmaveer *et al.*, 2007; Kim *et al.*, 2014). The results of the present work agreed with those reported by Ayo *et al.* (2007), who reported that using ingredients in formulations that cause an increase in fat content and a decrease in moisture content resulted in an increase in hardness due to the concentration of available muscle protein, which resulted in tougher structures. Together with the loss of humidity that causes hardness in sausages, oxidative damage to proteins affects protein solubility, which can lead to cross-linking, increasing hardness (Karel *et al.*, 1975).

Although protein oxidation was not assessed in the present work, we did observe an increase in lipid oxidation levels as the liver content in the formulation increased. Lorenzo *et al.* (2014) reported a significant correlation between lipid and protein oxidation, for which it was hypothesised that the increase in the hardness of frankfurters may also be due to protein oxidation. According to Ganhão *et al.* (2010), protein oxidation contributes to the hardness of meat products through the formation of protein carbonyls, a loss of protein functionality, and the formation of cross-links between proteins during cold storage. A relationship between protein oxidation and increased hardness has been observed in frankfurters (Estévez *et al.*, 2005), dry-cured mutton ham (Guo *et al.*, 2021), and meatballs (Kotecka-Majchrzak *et al.*, 2021).

Thiobarbituric acid-reactive substances and retinol content

The thiobarbituric acid reactive substance (TBARS) values of frankfurter-type sausages formulated with different pork liver contents during storage are shown in Figure 2A. The oxidation values of the frankfurters were affected by the addition of different pork liver levels and cold storage at 2°C for 60 d. The lipid oxidation values in the control group remained below 0.3 mg MDA/kg during the entire storage period ($p > 0.05$). Adding more than 8% liver to the formulation increased the oxidation levels from day 12 of storage. Adding 4% pork liver to the formulation resulted in oxidation values higher than 0.4 mg MDA/kg from day 36 of storage, values higher than those of the control ($p < 0.05$). The formulation with 12% pork liver presented values close to 0.6 mg MDA/kg from day 36 of storage, and

higher than 0.7 mg MDA/kg at the end of the storage period, values much higher than those found in the other formulations ($p < 0.05$). In all formulations, a gradual increase in oxidation was observed as the storage period progressed.

An increase in lipid oxidation was observed when the amount of liver in the frankfurter increased, probably due to the high iron content in the liver since it can generate reactive oxygen species. Likewise, free iron ions catalyse the decomposition of lipid hydroperoxides, forming peroxide and alkoxyl radicals that can initiate new chain reactions or further decompose to produce aldehydes and other secondary products that cause lipid oxidation (Carlsen *et al*., 2005; Orino and Watanabe, 2008). Even though the addition of the liver caused an increase in the degree of lipid oxidation in the frankfurter, the values of all the treatments remained below 1 - 2 mg MDA/kg meat after 60 days of storage at 2°C. These values are considered the acceptable thresholds for rancidity in meat products (Devatkal *et al.*, 2004; Munsu *et al.*, 2021).

Endogenous enzymes and metals in the liver accelerate lipid oxidation during the cold storage of liver-based meat products; for that reason, researchers have explored various strategies to mitigate oxidative damage, yielding promising results. Choe *et al.* (2019) found that the use of natural powders derived from green tea, lotus leaves, and kimchi effectively extended the shelf-life of sausages with 20% chicken liver. Similarly, Pateiro *et al.* (2015) reported a decrease in lipid oxidation in pork liver pâté containing tea and grape seed extracts compared with the control treatment after 24 weeks of storage at 4°C. Moreover, Chernukha *et al.* (2023) used *Allium cepa* husk extract as a natural antioxidant to reduce lipid oxidation of pâté during cold storage. Considering these studies, for future research, the use of natural antioxidants could be an excellent strategy to reduce lipid oxidation in frankfurters containing up to 12% pork liver.

The retinol contents in the frankfurter-type sausage with added pork liver are presented in Figure 2B. The retinol was affected by the addition of different pork liver levels ($p < 0.05$), and not by the storage time at 2°C for 60 days ($p > 0.05$). The retinol content in frankfurters without added pork liver was lower than 0.2 mg retinol per 100 g of sample during the first 12 days of storage, with an increase (approximately 0.4 µg retinol per 100 g of sample) after day 36 ($p > 0.05$).

The retinol content increased in frankfurters when a greater percentage of pork liver was added to the formulation, with the highest retinol content being observed in the 8 and 12% pork liver formulations. Adding 4% pork liver to the formulation resulted in a retinol content higher than 200 µg retinol per 100 g of sample, while adding 8 and 12% resulted in higher than 800 and 900 µg retinol per 100 g of sample, respectively. The addition of pork liver increased the retinol content as the percentage of the liver in the formulation increased, but the retinol content did not change during the 60 days of storage period in each formulation. Kim (2011) reported that internal organs such as the liver contain more vitamin A than muscle tissue. Similarly, Seong *et al*. (2014) reported that the liver contains a high concentration of vitamin A (57,406.68 µg RE/100 g). In the specific case of pork liver, Kim (2011) reported that pork liver contains a higher vitamin A content than beef and lamb liver. Therefore, the high amount of retinol in pork liver makes it an excellent option for addition into foods aimed at counteracting vitamin A deficiencies in the population.

Conclusion

The present work demonstrated that adding pork liver to formulations of traditional meat products, such as frankfurters, considerably increased the retinol, iron, zinc, and selenium contents. Importantly, pork liver did not negatively affect the protein/fat content of the new functional frankfurter-type product. However, it slightly affected the instrumental colour and texture, especially at high levels (12%). The changes in these quality parameters were greater after 60 d of cold storage. Likewise, high addition of pork liver in the meat formulation accelerated lipid oxidation during cold storage; for this reason, natural antioxidants are recommended to avoid an increase in quality deterioration. The present work constituted the initial stages in the research needed to develop a functional food for real-world applications. The results of the present work would contribute to the scientific knowledge needed to develop a new product that addresses micronutrient deficiencies in vulnerable population groups. However, studies that address sensory aspects, the use of natural antioxidants, and bioaccessibility and bioavailability studies of the active components, among others, are still necessary.

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