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Fortifying pork liver mixture: preparation and physicochemical characteristics - Part 1

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Abstract

Micronutrient deficiency is a worldwide health problem, especially in developing countries. A survey conducted over the last decade has shown that approximately half of all Brazilian preschoolers have iron-deficiency anemia, representing a severe public health problem. Therefore, the aim of this research was to evaluate the physicochemical and microbiological attributes and especially the iron level of a fortifying mixture (FM) containing pork liver prepared in powder form. Centesimal composition, fatty acids, minerals and microbiological evaluation were determined. The results obtained showed that this is a product of good microbiological quality with a high protein (47.34%) and lipid (28.29%) content. Monounsaturated fatty acids represented 8.13% of the total fatty acids, the main one being oleic acid (C 18:1 ω9) at 7.57%, corresponding to 93.22% of the total monounsaturated fatty acids. Regarding mineral content (5.18%), the FM proved to be an important source of iron (23.8 mg/100 g), also containing expressive amounts of other minerals, zinc in particular (9.97 mg/100 g). The results showed that FM has characteristics that make it an excellent fortifying food to be used in school meals mainly in soups, creams, cooked meats and especially in beans, a preparation that is already part of the Brazilian food habits since childhood.

Keywords: iron deficiency; micronutrient fortification; dietary strategies; school meals.

Practical Application: The fortifying pork liver mixture (FM) prepared in this study is a product with a high protein content and an important source of iron, also containing expressive amounts of other minerals, zinc in particular. By being in powder form the FM can be easily added to soups, creams, cooked meats, beans and other foods. These characteristics render the FM an excellent fortifying food with a great potential for use in school meals.

1 Introduction

Iron deficiency and iron-deficiency anemia are terms used as synonyms although they do not represent the same condition. According to the World Health Organization (2001), iron deficiency is the result of a long-term negative iron balance, with anemia being the most severe stage of this deficiency.

Iron-deficiency anemia affects about 1.62 billion people worldwide, with preschoolers being the most affected group (47.7% according to the World Health Organization, 2008). The estimate is that about 4.8 million Brazilian preschoolers were affected by iron-deficiency anemia in the early 2000's, representing about half of all preschoolers and a consequent severe public health problem (Brasil, 2004; World Health Organization, 2008). Among children, the consequences can be irreversible, affecting cognitive, motor and social development and impairing learning and immunity, among other physiological processes essential for life (Bortolini & Vitolo, 2010; Horton & Ross, 2003).

Iron supplementation, a diversified diet and food fortification are strategies for the prevention and treatment of anemia, the latter strategy being considered to be the best way to increase the intake of mineral iron by being sustainable, simple and of low cost (Davidsson & Nestel, 2004; Schafer & Assunção, 2011).

Home food fortification with powders containing various micronutrients has proved to reduce anemia and iron deficiency in children 6 to 23 months of age. In 2011, the World Health Organization (WHO) emitted guidelines about the utilization of these preparations in foods consumed by infants and children 6 to 23 months of age (De-Rigel et al., 2013; World Health Organization, 2011).

Thus, the dietary approach using fortifying mixtures (FM) is considered to be a safe strategy for the prevention and combat of iron-deficiency anemia by guaranteeing a supply of moderate iron amounts accompanied by greater amounts of other nutrients equally important for the reversal of the disease (Allen, 2008). In addition, the incorporation of FM into food preparation preserves food practices by favoring the use of food, has a non-discriminatory characteristic by being adopted by all children covered by the School Feeding Program, and involves fewer risks of oscillations in the exposure to high or low doses due to forgetfulness or mistakes in the offer of the supplement (Adu-Afarwuah et al., 2008).

Among the foods that may be part of this or other fortification programs, the use of pork liver FM would be an interesting alternative since the liver if a low-cost, iron-rich byproduct

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of swine slaughtering. In addition, according to the Annual Report of the Brazilian Association of Pork Meat Producing and Exporting Industries (Associação Brasileira da Indústria Produtora e Exportadora de Carne Suína, 2013), Brazil is the third largest producer and the fourth largest exporter of pork meat in the world.

Since the main causes of iron-deficiency anemia are an insufficient intake of iron-containing foods and insufficient absorption of ingested iron, the objective of the present investigation was to study the preparation and the physicochemical attributes of a pork liver FM with more aggregated value as an alternative contributing to a better diet regarding the prevention and control of iron deficiency in children.

2 Methods

A pâté containing a considerable amount of pork liver was prepared and lyophilized as an FM containing predetermined iron levels (1.5 to 2.5 mg/day) to be added to food preparations, respecting natural limits due to its addition such as changes in flavor, color and aroma.

Various formulations were tested in preliminary assays in order to establish the proportions of ingredients that would result in a product of good sensory quality (Table 1).

2.1 Preparation of the fortifying mixture

The flow diagram of the process of FM preparation is illustrated in Figure 1.

2.2 Assessment of the fortifying mixture

Centesimal composition

Moisture (Instituto Adolfo Lutz (2008), mineral matter, protein, lipids (Horwitz, 2000) and carbohydrate (as the difference) were determined.

Fatty acid composition

Fatty acid composition was determined by gas chromatography after sample esterification by the method of Hartman & Lago (1973). Fatty acids were determined by comparing the time of fatty acid retention of the sample to that of standards. Fatty acids were quantified by area normalization and the results are expressed as g/100 g sample for each fatty acid.

Table 1. Quantities of the ingredients used in the formulation of the fortifying mixture.

Ingredients	Quantity (g/100g)
Porkliver	54.30
Onion	17.70
Garlic	0.70
Skimmedmilk cream	25.70
Soyoil	1.10
Salt	0.50
Total	100.00

Determination of minerals

Calcium, copper, iron, phosphorus, magnesium, sodium, and zinc were determined according to AOAC procedures (Horwitz, 2010).

Microbiological analysis

Salmonella sp., *S. aureus*, heat-tolerant coliforms, *E. coli*, molds, yeasts, and sulfite-reducing *Clostridium* (46 °C) were determined by the methods described by Silva et al. (2010).

3 Results and discussion

3.1 Centesimal composition

The centesimal composition of the fortifying mixture (FM) is listed in Table 2.

The protein content value (49.15% DB) was close to that reported by Martin et al. (2009), who obtained 48.82% (DB) for a pork liver pâté. Thus, the FM was characterized as a product with a high content of protein of animal origin, with a good essential composition of amino acids recognized as being biologically available.

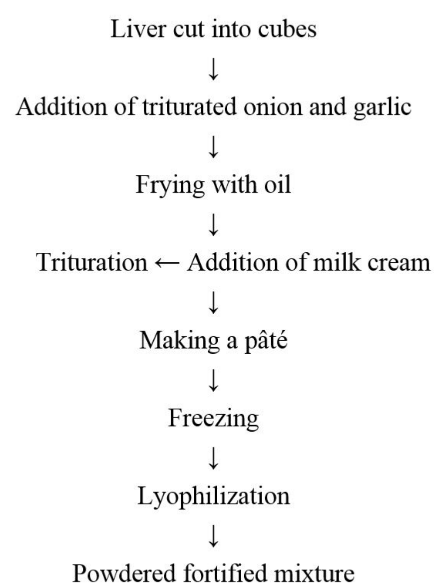

Figure 1. Flow diagram of the process of FM preparation.

Table 2. Centesimal composition of the fortifying mixture (FM). Data are reported as mean + SD.

Determination (g/100g)	Fortifying mixture	
	Moist base (MB)	Dry base (DB)
Moisture	3.68 (0.12)	- - -
Ashes	5.18 (0.03)	5.37 (0.03)
Protein	47.34 (0.13)	49.15 (0.15)
Lipids	28.29 (0.08)	29.37 (0.23)
Carbohydrate[1]	15.51 (0.07)	16.11 (0.12)

[1]Determined as the difference.

The lipid content of the FM (29.37% DB) was similar to the value reported by Beldarraín et al. (2007), in a study of pâtés of different flavors (32.09% DB). However, Dalmás et al. (2011) working with different types of pâtés, obtained lipid contents of about 70%, higher than those detected in the present study.

Comparatively, the high protein content associated with lower lipid content causes the present FM to be a product of potential use in school meals for the combat of nutritional deficiencies.

The mineral matter values reported by D'Arrigo et al. (2004), when working with different types of pâtés ranged from 4.29 to 6.94% DB. Thus the mineral matter content of the present FM (5.37% DB) was within the range reported by the cited authors.

Regarding carbohydrates, the value (10.05%, DB) reported by Dalmás et al. (2011) was below the value detected in the FM studied here (16.11%, DB).

3.2 Fatty acid composition

The fatty acid composition of the lipid fraction of the FM is listed in Table 3.

The data show that the main fatty acids present in the FM were myristic (C 14:0), palmitic (C 16:0), stearic (C 18:0), oleic (C 18:1 ω9) and linoleic (C 18:2 ω6) acids, representing together 85.68% of the total amount of fatty acids present in theFM. Comparatively, the presence of the remaining fatty acids in the FM was nonsignificant in terms of quantity.

Regarding the saturated fatty acids (15.98 g/100 g, myristic (C 14:0), palmitic (C 16:0) and stearic (C 18:0) fatty acids represented, together, 90.05% of the total amount of saturated fatty acids present in the FM. Estévez et al. (2005), detected a total of 36.38% saturated fatty acids in pâtés with pork liver added, a value 127.66% above the one detected in the FM. The lower proportion of saturated fatty acids causes the present FM to be a healthier product because, according to Bragagnolo (2001), the saturated myristic (C 14:0), lauric (C 12:0) and palmitic (C 16:0) fatty acids are considered to be hypercholesterolemic, although palmitic and myristic acids have the highest atherogenic effect.

According to Sinclair (1993), stearic fatty acid (C 18:0) is immediately transformed to oleic acid (C 18:1) in the organism, without affecting cholesterol levels. Thus, if the quantity of stearic fatty acids is subtracted from the total saturated fatty acids in the FM, the total quantity of fatty acids is reduced to 10.75%, a relatively low value.

Table 4 lists the total fatty acids as a function of classification into groups (saturated, monounsaturated, polyunsaturated and *Trans*).

The total amount of monounsaturated fatty acids of the FM was 8.12% and the main component was oleic acid (C 18:1ω9) with 757%, corresponding to 93.22% of the total amount of monounsaturated fatty acids. Estévez et al. (2005) emphasized the high proportion of oleic acid and monounsaturated fatty acids detected in products elaborated from pork liver. The value of polyunsaturated fatty acids was 1.44 g/100 g, basically consisting of the omega 6 fatty acid (1.20%).

Table 3. Profile of the fatty acids present in the lipid fraction of the fortifying mixture.

Fatty acid	Fortifying mixture[1]	
	% area	g/100 g
C 6:0	0.44	0.12
C 8:0	0.36	0.10
C 10:0	0.99	0.27
C 11:0	0.10	0.03
C 12:0	1.39	0.37
C 13:0	0.07	0.02
N.I.	0.08	0.02
C 14:0	6.09	1.65
N.I.	0.60	0.16
C 14:1 ω5	0.40	0.11
C 15:0	0.73	0.20
N.I.	0.20	0.05
C 16:0	27.76	7.51
N.I.	1.07	0.29
C 16:1 ω7	1.20	0.32
C 17:0	0.90	0.25
N.I.	0.16	0.04
C 17:1	0.27	0.07
C 18:0	19.35	5.23
C 18:1 ω9*T*	2.35	0.64
C 18:1 ω9	28.00	7.57
C 18:2 ω6*T*	0.41	0.11
C 18:2 ω6	4.47	1.20
C 20:0	0.31	0.09
N.I.	0.08	0.02
C 20:1 ω11	0.13	0.03
C 18:3 ω3A	0.12	0.03
CLA[2]	0.28	0.07
N.I.	0.18	0.05
C 22:0	0.21	0.05
N.I.	0.14	0.04
C 20:4 ω6	0.77	0.21
C 24:0	0.30	0.09
C 24:1	0.09	0.02
Total	100.00	27.03

[1]Total lipids = 28,29%; [2]Total Conjugated Linoleic acid, Factor: 0.956 (CQ: 7943/2011)

Table 4. Fatty acids present in the lipid fraction of the fortifying mixture according to their groups.

Total fatty acids	Fortifying mixture	
	% Area	g/100 g
Saturated	59.00	15.95
Monounsaturated	30.09	8.13
Polyunsaturated	5.36	1.45
Omega3	0.12	0.03
Omega6	5.24	1.42
Trans + CLA[1]	3.04	0.82
NI[2]	2.51	0.68
Total	100.00	27.03

[1]CLA is included in the *Trans* group; [2]NI = not identified.

In the FM, the percentage of unsaturated (monounsaturated + polyunsaturated) and saturated fatty acids (minus stearic acid) was35.4 and 39.8%, respectively.

Due to their importance, lipids are among the bioactive compounds (functional ingredients) receiving greater attention (particularly in terms of quantity and quality) regarding the development of healthier meat-derived products (Jiménez-Colmenero et al., 2010).

For this reason, a constant concern regarding the formulation of meat-derived products is to use ingredients that will establish the quantity and the profile of fatty acids in order to reach a more convenient composition in terms of nutritional quality. The quantity of fat in the formulation of meat products, the level of saturation and the fatty acid composition are factors of major importance for the quality of the product and the health of the consumer (Zorba & Kurt, 2008).

3.3 Mineral determination

Table 5 presents the mean results of three replicates of mineral determination in the fortifying mixture.

Among the byproducts of animal slaughtering, regardless of species (cattle, swine, sheep, fowl etc.) or race, the liver represents an important source of minerals, although the mineral profile may vary as a function of genetic lineage, race, feeding, management etc. (Tomović et al., 2011).

The high iron content (23.8 mg/100 g) of the present FM is due to the fact that the mixture contains large amounts of pork liver, a meat byproduct with high vitamin and mineral concentrations. This is in contrast to other cuts usually employed for the fabrication of meat products and derivatives, since the iron content of the liver (16.65-30.93 mg Fe/100 g) is significantly higher than that of the animal's meat (Tomović et al., 2011). Also, by being a lyophilized product, the FM contains concentrated nutrients due to the removal of water. The iron content data obtained by Dalmás et al. (2011) for 3 pâté formulations containing goat liver, blood and meat (6.48, 7.92 and 9.99 mg Fe/100 g) were 2.4 to 3.7 times lower than those obtained for the present FM.

In turn, Hallberg et al. (2003) stated that the addition of red meat in powder form to baby food during breastfeeding significantly increased iron absorption up to 12 months of age and that the addition of ascorbic acid to powdered red meat can be a viable option to satisfy the high requirement of iron needed during the breastfeeding period. Based on the reports by these authors, we may predict that the FM, in addition to making available a larger amount of iron, should also increase iron absorption.

Table 5. Mineral content of the fortifying mixture. Data are reported as mean + SD.

Minerals	Fortifying mixture
(mg/100g)	Mean + SD
Calcium	109 (1)
Copper	1.04 (0.01)
Iron	23.8 (0.4)
Phosphorus	858 (13)
Magnesium	48.8 (0.2)
Sodium	909 (11)
Zinc	9.97 (0.06)

According to Domene (2009), preschoolers require 10 mg Fe/day to satisfy their growth and development needs. On the basis of this statement and considering the insertion of the FM into school meals, it is necessary to add 6.3 g FM to 60 g beans (an amount equivalent to one portion for a child of this age) in order to satisfy 15% of the daily nutritional requirements for preschoolers, as determined by the National School Feeding Program (Brasil, 2013).

Zinc is involved in several biochemical functions since it is present in more than 300 enzymes, including those of the central nervous system. This mineral acts on cell division, on gene expression and on the physiological processes of growth and development (Fidelis & Osório, 2007). The value present in the FM (9.97 mg/100 g) corresponds to twice the value reported by Dalmás et al. (2011) (5.05 mg/100 g, DB).

Since the daily zinc requirement of preschoolers is 10 mg (Domene, 2009) the addition of 6.3 g FM previously considered to satisfy 15% of the daily iron requirements would satisfy 6.3% of the daily zinc requirements.

The magnesium content of the FM (48.8 mg/100 g) was slightly higher than that reported by Dalmás et al. (2011) (43.71 mg/100 g, DB).

The sodium value obtained for the FM (909 mg/100 g) was predominantly due to the addition of sodium chloride to the formulation. In Brazil, sodium consumption has always been above recommended requirements, being part of the Brazilian eating habits. Although today we are aware of the problem represented by excessive sodium consumption, reversing this tendency has not been an easy task because of the influence of salt on the flavor of food. However, the current trend is to reduce salt consumption because of its influence on chronic-degenerative diseases. With the use of the FM, a meal will still require the addition of salt as a condiment although, in the portion suggested (6.3 g), the amount of sodium will only be 58 mg, or the equivalent of 0.16 g salt.

Copper, a heavy metal, when present in quantities exceeding the maximum intake limit established by Public Health Organs is considered to be toxic since it may cause severe neurological problems, gastric disorders and many other symptoms. Tomović et al. (2011) reported that the mean liver copper content was about 60% higher than the value present in the FM (1.04 mg/100 g). Since the maximum copper content permitted by law is 3ppm (Brasil, 1998) and considering the addition of 6.3 g FM per portion of beans (60 g) to the school diet, the quantity of copper supplied by the FM would correspond to 21.8% of the maximum limit permitted.

The phosphorus content of pork liver is of the order of 375 mg/100 g, a value considered high when compared to that of pork meat (227 mg/100 g). Thus, products containing liver have higher phosphorus content than products that do not contain liver. For this reason, the phosphorus content detected in the FM (858 mg/100 g) was 55.4% higher than that detected by Dalmás et al. (2011) (552 mg/100 g, DB).

The calcium content of the FM (109 mg/100 g) was much higher than that obtained by Dalmás et al. (2011) (24 mg/100 g, DB).

Thus, the FM, by being in powder form and requiring no cooking or heating, can be easily added to soups, creams, cooked meats, and mainly beans, a preparation that is already part of the Brazilian diet since childhood (Assunção & Santos, 2007). With these characteristics, the FM represents an excellent fortifying food to be used in school meals. However, the quantities of FM to be added should be calculated for any preparation to be considered.

Zancul (2004) pointed out that food fortification is recognized for its efficiency in improving, preventing and even eradicating diseases caused by nutrient deficiency.

3.4 Microbiological analysis

Microbiological analysis of the FM regarding the presence of Bacillus cereus, Salmonella sp., coliforms at 45 °C and total and fecal coliforms (Petrifilm) yielded results that agree with the values established by the RDC nº 12 of January2001 (Agência Nacional de Vigilância Sanitária, 2001) showing that the mixture is adequate for consumption.

4 Conclusion

The pork liver formulation resulted in a powdered product denoted Fortified Mixture (FM) which showed good microbiological quality, thus representing an important source of iron for children's feeding. In addition, the presence of other nutrients observed suggests that the present product can contribute in an expressive manner to the daily requirements of zinc, phosphorus, magnesium and copper.

Studies of the iron bioavailability and biological utilization of the FM will complement the present investigation and may expand the understanding of the potential of this product as a food supplement.

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