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Antioxidant properties of *paratha* type flat bread enriched with white mulberry leaf extract

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The pace of modern life promotes the occurrence of diseases of affluence. Functional food may be useful in the prevention of different diseases and is becoming an alternative to food products available on the market. Far East Medicine as well as Indians had used white mulberry leaves as important source of biocompounds. Nowadays people ought to remember about this plant and try to use it in every-day diet. Bread is an essential element of the meals in middle Europe, however flat bread is not widespread in this part of the world. The design and properties of flat bread enriched with mulberry leaves were the overriding objectives in the study. The centuries-old use of mulberry leaves in alternative medicine suggested a potential positive outcome. In designed five variants of *paratha* the stability and content of polyphenols after thermal process and antioxidant properties were tested. It was showed that extract obtained on a semi-technical scale is rich in polyphenols which results in antioxidant activity of final functional food product-*paratha*. The addition of the extract significantly increased the health-promoting qualities of *paratha* and makes bread be desired by the consumers.

Keywords: Antioxidant properties, Traditional plant, Flat bread, Functional food, Polyphenols

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The pace of life in the modern world has greatly increased. This has a huge impact on diet and lifestyle of residents of developing countries. Such behaviour has a significant impact on the development of so-called lifestyle diseases[1,2,3]. Therefore, consumers are looking for ways to prevent diseases. Using plant materials with pro-healthy activity in human nutrition is one of them. Functional food is a great response[4,5]. Definition of functional food was specified[6,7]. Its pro-health status is based on the presence of bioactive substances stimulating metabolism, and preferably appropriate amounts of the individual ingredients for a particular organism[8,9]. The addition of special plant material is decisive for their specific functionality. Many plants exert positive effects on blood sugar, insulin sensitivity, and dyslipidaemia in human organisms[2]. White mulberry (*Morus alba* L.) belongs to an extensive group of plants, the use of which in Far Eastern Medicine is already a centuries-old tradition[10,11,12]. The leaves have been used as a therapeutic agent. Nowadays, in alternative medicine also fruits as well as root bark and young sprouts are used[13,14,15]. Recent studies showed that the wealth of chemical compounds contained in mulberry leaves is so extensive that it can successfully support the prevention of lifestyle diseases[12]. In Asia, there is every day food products which utilize those properties of plants considered to be medicinal[16,17]. Flat loaves of bread are an example constituting a common element of Indian meals. *Paratha, paratta, parantha* are made from wheat flour and fried with ghee. *Paratha* types differ depending on geographic region, although *paratha* itself is probably derived from the Punjab. *Paratha* often contains spices and mulberry leaves[18]. In Poland, other types of bread (wheat bread and rye sourdough bread) prepared with yeast and lactic acid bacteria, are widely consumed. Our team have already made an attempt to enrich such breads with an extract of mulberry leaves. Results are in press. The possibility of introducing *paratha* into a range of functional food may be an interesting alternative. For this reason, the overriding objective of the study was to produce a new product for the Polish market with health-promoting properties and to establish the best level for this addition of mulberry leaf extract (MLE) to bread. An additional objective was to examine the stability of polyphenols and antioxidant activity of designed flat bread.

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Methodology

White mulberry leaves

The leaves of the Polish variety Wielkolistna zolwinska were collected by hand from the experimental plantation of the Institute of Natural Fibres and Herbal Plants (Petkowo near Poznan, Poland) in July, 2011. The leaves were stored at +5 °C before use.

Preparation of dried mulberry leaves

Mulberry (*Morus alba* L.) leaves were dried in a drying chamber (30 °C) and used to extract preparation. Dried leaves as a component of flat bread were ground (3000 rpm/min, 2min) using mill (Retsch GM200). Dried leaves were stored closed at room temperature.

Preparation of mulberry leaf extract

An aqueous extract of leaves was obtained in semi-technical scale by a combination of processes: continuous extraction (water at 80-90 °C using the counter current method; shredded leaves: water 1:10 (w/w) to obtain 2-4% dry matter after filtration); vacuum concentration (in periodic spherical evaporators at 75 °C, pressure 0.6-0.8 atm.); spray drying (air 180-190 °C to obtain powder of 96-98% of dry matter).

Flat bread (*paratha*) components

A blend of herbs de Provence (rosemary, basil, thyme, sage, peppermint, summer savory, oregano, marjoram) and blended curry spice were from Kamis, Poland. Wholemeal wheat flour, *graham* 1.85% ash and white wheat flour, 0.5% ash were from Lubella, Poland. Iodized salt and rapeseed oil were from Kujawy, Poland.

Paratha production process

Five different variants of the dough were prepared according to MLE and wholemeal wheat flour content (Table 1). The dough was made after modification of many Indian recipes [I, II, III]. The production process was as follows: ingredients were mixed with water; the dough was kneaded by hand to obtain a uniform consistency and covered with cotton cloth, left for 30min; divided into 8 pieces and rolled out into a thin cake, then smeared with oil and folded in half to get a triangle and fried on a Teflon-coated pan (180 °C/5min).

Dry matter assay

The assay was based on a weighting out of 1g of sample (accuracy of 0.001), and then drying in a laboratory oven (Herbatherm, Thermo Scientific) at 105 °C to constant weight. The results were as % of water content[19].

Fat content assay

The assay was based on a weighting out of 5 gm of sample into a thimble, drying, and continuous extraction with petroleum ether in a Soxhlet extractor (Foss Tecator). The flask with a mixture of fat and ether is subjected to evaporation, and then dried[19].

Protein content assay

Determination of protein using the Kjeldahl method (Kjeltec 2200, Foss Tecator) consisted of three steps: mineralization, distillation and titration. Mineralization of nitrogen-containing organic substance occurs by combustion of 1 gm of sample in presence of concentrated ardiovas acid at the boiling point of acid[19].

Ash content assay

The method was based on the combustion of 1gm of sample in a flask at 550 °C in a muffle furnace (Herbatherm, Thermo Scientific) for about a day[19].

Preparation of extracts of dough and paratha

Extracts of dough and *paratha* were made using the Siger method[20] with modifications: 10 gm of the sample shredded (3000 rpm/min, 1.5min) in a laboratory mill were put into conical flasks, filled with 80% methanol, shaken in a water bath (50°C/30min), and the extract was pooled. After four-time extraction, extract was filtered (Whatman1 filter + Büchner funnel) with an aspirator.

Table 1— The composition of different variants of *paratha*

Paratha –type	Ingredients [%]								
	Wholemeal wheat flour	White wheat flour	Tap water	Salt	Dried mulberry leaves	Mulberry leaf extract	Curry	Herbs de Provence	Rapeseed oil
A	43.7	14.6	29.3	0.1	2.0	0.0	0.1	1.5	7.5
B	41.7	14.6	29.3	0.1	2.0	2.0	0.1	1.5	7.5
C	40.8	14.6	29.3	0.1	2.0	3.0	0.1	1.5	7.5
D	39.8	14.6	29.3	0.1	2.0	4.0	0.1	1.5	7.5
E	38.8	14.6	29.3	0.1	2.0	5.0	0.1	1.5	7.5

Total phenolic content assay

The total phenolic compounds content in extracts was spectrophotometrically determined with the Folin–Ciocalteu procedure by reading the absorbance at 765 nm against a methanol blank. As a control a solvent of sample was used. Results were expressed as $mg*g^{-1}$ of dry matter for gallic acid (GAE)[21].

DPPH scavenging activity assay

The method was based on the spectrophotometric measurement of changes in absorbance at λ=517nm[22]. As a blank, $DPPH^{\bullet}$ was replaced by methanol. As a control, a sample solvent was used instead of sample. A standard curve was prepared at Trolox concentrations: 5, 7.5, 10, 12.5, 15, 17.5, 20 μM/mL. The scavenging activity was showed as inhibition [%] result of hydrogen donor activity of the sample:

$$DPPH\ scavenging\ [\%] = 100 - \left[\frac{\left(Abs_{samples} - Abs_{blank}\right)}{Abs_{control}}\right]\times 100$$

$ABTS^{\bullet+}$ scavenging activity assay

The method was based on the spectrophotometric measurement of the change in absorbance at λ=734 nm[23]. A solution of $ABTS^{\bullet+}$ should be prepared the day before. Potassium persulfate ($K_2S_2O_8$) and $ABTS^{\bullet+}$ stock solution were mixed (1:0.5, v/v) to prepare the $ABTS^{\bullet+}$ solution. The mixture was allowed in a darkness for at least 12-16 hrs. Next day, methanol was used to dilute $ABTS^{\bullet+}$ solution to an absorbance of 0.700±0.020. A solvent of the sample was used as a control. The $ABTS^{\bullet+}$ scavenging activity was calculated as follows:

$$ABTS^{+}\ scavenging\ [\%] = \left[\frac{Abs_{control} - Abs_{samples}}{Abs_{control}}\right]\times 100$$

Fe^{3+} reducing power assay

The method was based on the colorimetric measurement of the concentration of Prussian blue which is formed in an Fe^{2+} reaction derived from the reduction of Fe^{3+} [24]. Absorbance was measured at λ=700nm.

Fe^{2+} chelating activity assay

The method was based on the measurement of the absorbance in a solution, in which the iron (II) is not bound with the sample in the reaction with ferrozine at λ=562nm[25].

Sensory evaluation of *paratha*

Evaluation of *paratha* was performed by 34 individuals in sensoric laboratory in equal ambient conditions, using two methods[26]: quality assessment method with a 10 cm linear scale, non-structured with no boundary marks (highlights: appearance, color, ardiov, smell, texture, overall rating); assessment of preferences using a 9-point hedonic scale with verbal expressions: like very much (9), like a lot (8), rather like (7), quite like (6), neither like, nor dislike (5), dislike a little (4), rather dislike (3), dislike very much (2), highly dislike (1). All individuals were instructed before analysis. Notes were listed on cards according to personal feelings. For quality assessment consumers had to mark graphically the level of desirability. Analysis of results was based on the distance measured on the axis of desirability for each highlight. For assessment of preferences (second method) consumers had to match one of 9 records for each sample. Each single sensoric evaluation lasted about 10-15 min. Samples were served at a ready-to-eat temperature.

Chemical reagents and standards

Reagents: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox, Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (Poland). All other chemicals used in the study were of analytical grade (POCH, Poland).

Statistical analysis

Results were reported as mean ±standard deviation of five independent experiments, and statistical comparison was evaluated by ANOVA analysis in Statistica9.0. $p<0.05$ was considered as statistically significant.

Results and discussion

Basic composition

Paratha is a specific variety of flat bread traditionally consumed in Asia with vegetable based fillings or cold sauces. *Paratha* is fried on a special frying pan (*tava*)[27]. The semi-technical scale extract of Polish mulberry leaves used in *paratha* production has previously been described[28]. Generally, the biologically active compounds from mulberry leaves especially affect antioxidant and cardiovascular properties, as it was well documented both in model systems and in animal studies[29,30,31,32]. In the study of *paratha* the protein, ash, fat and water content were analyzed (Table 2). The contribution of protein in

variants has been estimated at from 2.9% (A) to 4.3% (E). In variant C protein content increased to ~28%. This compound is defined as a plant protein which is highly desirable in the diet. Moreover, variants of *paratha* differed in terms of fat content, and its content ranged from 8.9-14.1%. This variability in the fat and water content could be associated with the difficulty encountered in the standardization of the entire production process on a small scale. The design and production of the product were conducted on a laboratory scale. The high ash content in *paratha* resulted from the presence of MLE, which contained about 23% ash[28]. According to the protein content upward trend was observed, as a result of addition of dried mulberry leaf to wheat flour. Dried mulberry leaves were added to dough at a constant level 2%. Such a small addition positively affected the palatability of the *paratha*.

Stability of polyphenols

Polyphenols constitute a large group of antioxidants. Due to widespread occurrence in plants, they constitute a significant component of human diet. Their consumption by an average consumer is estimated at up to 1gm/day[33,34]. Table 3 shows the changes in polyphenols content. In the dough, with an increasing addition of MLE, total polyphenol content increased. A similar effect was obtained by Lim *et al*.[35] in wheat bread enriched with 0-8% of turmeric powder, who showed that with increasing curcumin addition the content of polyphenols increased, regardless of baking process. In *paratha*, there were applied the curry spices with turmeric powder in the same amount (0.1%). The effect of curcumin on the antioxidant activity was taken into account (variant A). As a property of dried mulberry leaves, it was previously presented that air-drying temperature has an ambiguous effect on their antioxidant activity. Despite the high temperature dried leaves still showed high antioxidant activity and large amount of polyphenols[36]. In the studies, thermal processing of the dough caused a slight decrease in the polyphenol content (in B-E). The variant without extract (A) appeared to be an exception. Therefore, further researches are needed. It is known that phenolics are heat unstable compounds. High-temperature processes could damage then[37]. We observed that addition of MLE resulted in an increase in the polyphenol contents of dough. Unfortunately slight decrease in phenolics after frying was reported. The losses in the amount of polyphenols were generally proportional to amount of MLE. Nevertheless, mulberry leaf extract is a recommendable source of phenolics for this kind of bread.

The effect of the production process on the antioxidant activity

The ability to inhibit $ABTS^{\bullet+}$ and the scavenging activity of $DPPH^{\bullet}$ in dough and *paratha* were specified (Table 4). In the conducted studies,

Table 2-—The basic composition of the five variants of *paratha*, means ± SEM

Variant	Compound [%]			
	Water	Ash	Fat	Protein
A	26.9 ± 0.2	7.7 ± 0.1	12.3 ± 1.2	2.9 ± 0.1
B	22.2 ± 1.8	7.7 ± 0.0	14.1 ± 0.6	2.9 ± 0.1
C	24.1 ± 1.1	8.4 ± 0.2	12.6 ± 1.8	3.8 ± 0.2
D	22.9 ± 0.9	8.5 ± 0.0	8.9 ± 0.9	3.8 ± 0.0
E	21.9 ± 1.4	8.5 ± 0.1	11.7 ± 1.3	4.3 ± 0.0

Paratha without (A), with 2%(B), 3%(C), 4%(D) and 5% (E) of MLE.

Table 3—The total polyphenol content of the dough and the *paratha,* means± SEM

Variant	Total polyphenol content [mg GAE/gm dry matter of]		Decrease [%]
	dough	*paratha*	
A	$2.32^{b} \pm 0.79$	$1.25^{a} \pm 0.05$	46.13
B	$2.57^{a} \pm 0.03$	$2.53^{a} \pm 0.03$	1.56
C	$2.82^{a} \pm 0.03$	$2.76^{a} \pm 0.08$	2.13
D	$3.52^{b} \pm 0.05$	$3.19^{a} \pm 0.02$	9.37
E	$3.81^{b} \pm 0.04$	$3.40^{a} \pm 0.07$	10.77

Paratha without(A), with 2%(B), 3%(C), 4%(D) and 5%(E) of MLE. ^{a,b}significant differences ($p \leq 0.05$) in horizontal pairs of each variant dough:*paratha*

Table 4— The ability of scavenging $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, means±SEM

Variant	$DPPH^{\bullet}$ scavenging activity [µM Trolox/g dry matter of]		Decrease [%]	$ABTS^{\bullet+}$ scavenging activity [µM Trolox/g dry matter of]		Increase [%]
	dough	*paratha*		dough	*paratha*	
A	$7.46^{b} \pm 0.03$	$5.35^{a} \pm 0.07$	28.29	$0.60^{a} \pm 0.08$	$0.82^{b} \pm 0.04$	36.67
B	$11.77^{b} \pm 0.11$	$7.69^{a} \pm 0.08$	34.67	$1.60^{a} \pm 0.08$	$1.68^{a} \pm 0.02$	5.00
C	$12.16^{b} \pm 0.06$	$9.29^{a} \pm 0.05$	23.61	$1.89^{a} \pm 0.09$	$1.93^{a} \pm 0.03$	2.11
D	$12.93^{b} \pm 0.08$	$9.92^{a} \pm 0.03$	23.28	$2.33^{a} \pm 0.09$	$2.48^{b} \pm 0.03$	6.44
E	$14.98^{b} \pm 0.12$	$10.41^{a} \pm 0.04$	30.51	$2.96^{a} \pm 0.07$	$3.00^{a} \pm 0.01$	1.35

Paratha without(A), with 2%(B), 3%(C), 4%(D), 5%(E) of MLE. ^{a,b}significant differences ($p \leq 0.05$) in horizontal pairs – dough:*paratha*

increases in the scavenging activity of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ were observed together with increasing addition of MLE to dough. Frying caused a reduction in scavenging activity of $DPPH^{\bullet}$ by about 23-35% and were similar with Katsube *et al.*[38]. While frying water losses caused by condensation together with the absorption of oil were expected to increase the polyphenols concentration. On the other hand, under such conditions oxidation occurs resulting in a loss of polyphenols. The balance, determined as the final antioxidant activity, was measured. Results of $ABTS^{\bullet+}$ test showed that the frying did not significantly affect the inhibit. Indeed, one could even notice a slight increase in it. This phenomenon can be explained by the formation of melanoidal compounds during the Maillard reaction. Maillard reaction products (MRP) can cause increasing $ABTS^{\bullet+}$ inhibition[39,40,41]. This is particularly evident in variant A (36% reduction). Both $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ tests allow the determination of the antioxidant activity. The difference between these tests is the antioxidant system to which it is applicable. The $ABTS^{\bullet+}$ test is based on cation radical, which is applicable to hydrophilic and lipophilic systems, whilst the $DPPH^{\bullet}$ assay refers to the radical dissolved in organic media and is applicable to hydrophobic antioxidant systems. In the study, all variants of enriched *paratha* demonstrated the ability to chelate Fe^{2+} and Fe^{3+} reduction (Table 5), like elsewhere[42]. Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free-radical chain and donating a hydrogen atom[43]. This is a result of the presence of compounds that are electron donors and that can react with free radicals, for example polyphenols from mulberry leaves, which interact in many ways as strongly reducing[44,45]. The result was an increase in reducing properties in *paratha* with MLE. The ability to chelate Fe^{2+} was in Table 5. The chelating activity was observed in all types of *paratha*, and in dough (D, E). The addition of MLE resulted in a significantly chelating property increase (4-5 times higher ability) in *paratha*. Probably, the addition of MLE was too small in A, B, C dough. Iron is one of the redox active metals which possess the ability to produce reactive radicals which leads to oxidative stress. Some active compounds are capable of forming complexes. As a result, chelation of iron prevents its participation in redox reactions[46]. Differences between dough and *paratha* may be caused by MRP, which can inhibit oxidation by metal chelation[47]. In MLE we can find metal-binding compounds[36]. The *paratha* may protect biomolecules against oxidative damage – extremely important in incidence of lifestyle diseases.

Sensory evaluation of the designed flat bread

Paratha was produced in 5 variants with different amounts of MLE replaced the wholemeal flour. The addition of the MLE was estimated at the level of 0-5% (w/w). MLE provided a specific flavor and smell for the product, which was masked by curry and herbs de Provence. These spices increased the antioxidant levels in the final product and also affected the color (yellow). Wholemeal flour also increased the nutritional value of product[48,49]. *Paratha* was evaluated by a group of 34 consumers (Fig. 1). A linear 10 cm scale aimed to show the desirability and the quality of six attributes of products. However, the 9–point hedonic scale was to illustrate the desirability for each variant of *paratha* by fitting the verbal expressions to scale[26]. The consumer evaluation showed the greatest overall desirability for *paratha* with 3% of MLE. The highest scores for appearance and color were reported for *paratha* with 2 and 3% of MLE, and the lowest for *paratha* with the highest addition and without MLE. It can be explained by the difference in the color–yellow A and dark brown *paratha* E. Thus, it can be seen that the addition of

Table 5—The ability to Fe^{3+} reduction and to chelate Fe^{2+}, means±SEM

Variant	Fe^{3+} reduction [A_{700nm}]		Increase	Chelating activity [%]	
	dough	*paratha*	[%]	dough	*paratha*
A	$0.208^{aA} \pm 0.008$	$0.221^{bA} \pm 0.003$	6.25	$0.000^{aA} \pm 0.000$	$11.238^{bA} \pm 0.500$
B	$0.243^{aB} \pm 0.002$	$0.247^{bB} \pm 0.001$	1.65	$0.000^{aA} \pm 0.000$	$40.631^{bB} \pm 0.482$
C	$0.251^{aC} \pm 0.001$	$0.256^{bC} \pm 0.002$	1.99	$0.000^{aA} \pm 0.000$	$42.335^{bC} \pm 0.401$
D	$0.275^{aD} \pm 0.003$	$0.283^{bD} \pm 0.003$	2.91	$6.606^{aB} \pm 0.445$	$50.935^{bD} \pm 0.659$
E	$0.289^{aE} \pm 0.007$	$0.293^{aE} \pm 0.001$	1.38	$15.517^{aC} \pm 0.680$	$53.926^{bE} \pm 0.631$

Paratha without (A), with 2%(B), 3%(C), 4%(D) and 5% (E) of MLE. Significant differences ($p \leq 0.05$) in horizontal pairs – dough: *paratha* (^{a,b}) or in vertical groups – dough or *paratha* (^{A,B,C,D,E})

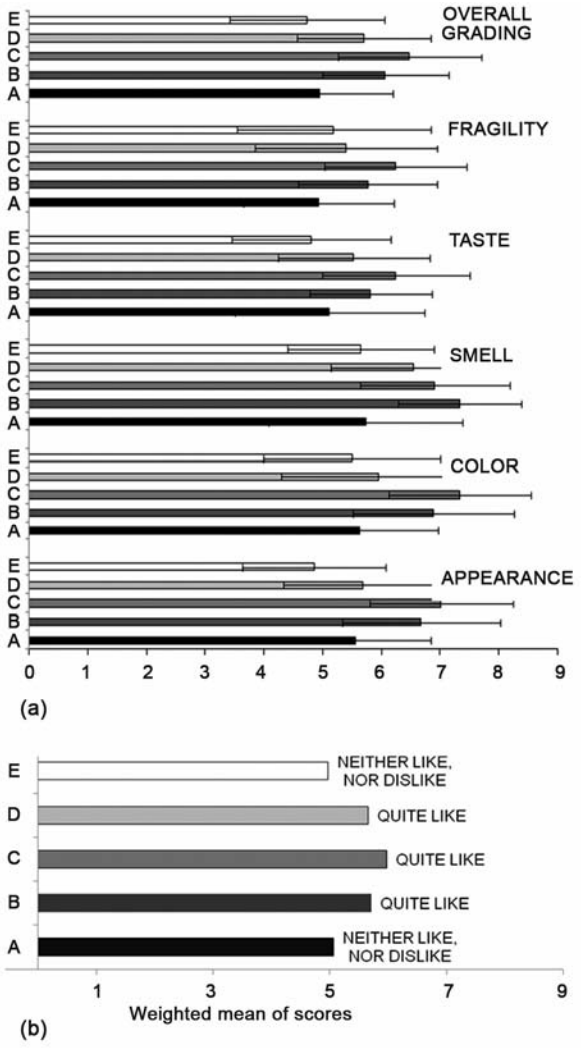

Fig 1—The values obtained for quality and desirability of appearance, color, smell, taste, fragility and overall grading on a linear scale (a) and averages values of desirability of variants of *paratha* (b). *Paratha* without MLE (A); *Paratha* with 2% MLE (B), 3% MLE (C), 4% MLE (D) and 5% MLE (E).

MLE affected, to some extent, the improvement of color desirability and appearance of the product. Using natural antioxidants in food production is becoming more popular[50], as well as interaction of medicinal plants with their bioactive compounds used as drugs are explored[51]. In this study it was shown that an unknown type of bread enriched with natural and cheap plant source of bioactive compounds can be accepted also by European consumers.

Conclusion

The applied frying process had a destructive influence on the polyphenols, the content of which in the final product was statistically significantly lower. Technological processing of *paratha* production effected a reduction in test with DPPH$^{\bullet}$ from about 1/3 to ¼ in relation to the value reported before process. Statistically significant increases were observed in ABTS$^{\bullet+}$ test after thermal process in comparison to dough. After technological process, the chelating properties of Fe^{2+} appeared and the ability to reduce Fe^{3+} increased. Among five proposed types, *paratha* with 3% of MLE appeared to be the best in a sensory evaluation. Despite the lack of knowledge about *paratha* among consumers, it was well accepted, yielding average values of desirability at about 6 = quite like. The extract obtained on a semi-technical scale was successfully used as an additive to produce this kind of traditional functional food.

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