

Development of Nutritionally Enhanced Tortillas

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Abstract Large interest has recently risen in the development of “functional” foods, products that may provide a health benefit beyond the traditional nutrients. Foods rich in antioxidants and, simultaneously, characterized by a low glycemic index (GI), can reduce, through a double mechanism, the risk of increased postprandial oxidative stress, which is one of the constituent of the onset of several chronic diseases. Nutritionally enhanced tortillas were therefore developed by incorporating ingredients with well-documented nutritional functionality (carrots, soy, and wholemeal kamut) in a standard wheat tortillas formulation, in an attempt to create low GI and antioxidant-rich products while preserving sensory acceptability and physico-chemical properties. Five tortilla prototypes were developed and characterized for sensory acceptability, textural attributes, color, total antioxidant capacity, and *in vivo* GI. The simultaneous combination of carrot juice, soy, and wholemeal kamut resulted in a very interesting product that was not only the most acceptable by the consumers (although slightly harder than the standard control) but also showed the lowest GI and was relatively high in total antioxidant capacity.

Keywords Tortillas · Functional food · Glycemic index · Total antioxidant capacity · Physico-chemical properties · Formulation

Introduction

Observational and intervention studies have shown that several chronic diseases can be prevented by lifestyle measures, including changes in diet composition.¹ The interest of the food industry in producing food items able to improve the nutritional quality of the diet and to somehow preserve consumers from the risk of nutrition-related diseases is rising faster than ever. In particular, a huge interest is focused on what is defined as “functional food.” Foods can be regarded as “functional” if proven to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way relevant to improved state of health and wellbeing, reduction in risk of diseases, or both.²

Clinical trials and epidemiological studies have demonstrated an inverse correlation between the intake of fruits and vegetables and the occurrence of several chronic diseases.³ This protective effect is likely due to the high content, in plant-based foods, of an array of antioxidant compounds, including polyphenols, vitamins E and C, and carotenoids. These molecules could exert their beneficial effects by acting in several different ways,⁴ but their antioxidant activity against oxidative stress is still one of the most relevant mechanisms.^{5,6}

Low-glycemic index (GI) foods have been shown to positively affect lifestyle through various mechanisms, including the improvement of glucose tolerance⁷ and helping maintaining a better cognitive performance throughout the day.⁸

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A recent seminal paper⁹ has linked two functional characteristics (GI and antioxidants) of food and observed the relation between blood glucose physiological fluctuations and oxidative stress, another paramount factor involved in the development of chronic diseases. Thus, improving glucose control by a low GI diet could also ameliorate the systemic oxidative stress level in human subjects. In this context, the addition of antioxidants to a low GI product was considered as a winning strategy to additionally improve its potential health effect. This double-faced application was also reported by Jenkins et al.,¹⁰ where antioxidants, provided together with a low GI meal, were able to decrease oxidative damage to proteins to an even more remarkable level.

Taken together, these observations clearly suggest the development of foods rich in antioxidants and characterized by a low GI, able to reduce through this double mechanism the risk of increased postprandial oxidative stress, which is one of the constituents of the onset of several chronic diseases.

Tortillas are a very common food in Central and South America but are becoming an important snack or bread substitute all over the world. Tortillas are thin, unleavened flat bread, made from finely ground maize (corn) or wheat flour and are characterized by a flexible texture and easy handling of the product. Tortillas are a good reference food that could be reformulated in an attempt to enhance their nutritional properties without significantly affecting the physico-chemical characteristics and products acceptability.

The objective of this work was, therefore, to develop nutritionally enhanced tortillas by incorporating ingredients with well-documented nutritional functionality (carrots, soy, and wholemeal kamut) in a standard wheat tortilla formulation, in an attempt to create a low-GI and antioxidant-rich product while preserving sensory acceptability and physico-chemical properties.

Materials and Methods

Tortilla Formulation, Production, and Characterization

Five prototypes of tortillas, standard (STD¹¹), carrot enriched (CAR), soy enriched (SOY), kamut (KAM), and one containing simultaneously carrot, soy, and kamut (CSK), were produced according to the formulations shown in Table 1. All ingredients were obtained at a local supermarket, and mono-diglycerides (DANISCO A/S DK) were provided by Danisco.

Tortilla prototypes were produced with the following process: dry ingredients were mixed with a spatula (Kitchen Aid, St. Joseph, MI, USA) for 2 min at speed 3, then margarine was added and mixed with a hook for 8 min at speed 3, and finally, distilled water (and/or carrot juice) was

Table 1 Tortilla prototypes formulation

Ingredient (%)	STD	CAR	SOY	KAM	CSK
Wheat flour	60.0	60.0	41.0	–	–
Whole soy flour	–	–	17.0	–	17.0
Whole kamut flour	–	–	–	60.0	41.0
Mono and diglycerides	2.5	2.5	2.5	2.5	2.5
Salt	0.9	0.9	0.9	0.9	0.9
Leavening agent	0.9	0.9	0.9	0.9	0.9
Margarine	4.0	4.0	8.0	4.0	8.0
Wheat gluten	–	–	1.6	–	1.6
Distilled water	31.7	14.3	28.3	31.7	9.2
Carrot juice	–	17.4	–	–	18.9

added and mixed with a hook for 1 min at speed 3 and for 4 min at speed 4. The dough was allowed to rest for 5 min at 25°C, was manually divided into 40-g pieces that rested for additional 25 min (25°C), and were then laminated twice (Unika Storci, Italy) to obtain round tortillas (1.30±0.10 mm thickness). Tortillas were cooked at 350°C on a glass ceramic skillet (Schott Ceran, Germany) for 15 s, flipped, cooked for 10 s, flipped again, and cooked for 5 s. Tortillas were allowed to cool at room temperature prior to packaging in polyethylene pouches.

Tortilla composition was determined with the following methods: proteins (AOAC 950.36¹²), lipids (AOAC 935.38¹³), fiber (AOAC 985.29¹⁴), water (105°C, constant weight), ash (AOAC 930.22¹⁵), and carbohydrates (difference to 100%).

Sensory Analysis

Sensory acceptability was evaluated with a ranking test, with 18 semitrained panelists. Five pieces of tortillas (one per each prototype) were randomly presented to the panel that ranked them from the most preferred to the least preferred for the following attributes: texture, color, flavor, and overall acceptability. Data analysis was carried out as previously reported.¹⁶

Color Analysis

Brightness (L^*), redness (a^*), and yellowness (b^*) of each sample were measured using a colorimeter (CM 2600d, Minolta, Osaka, Japan) equipped with a standard illuminate D65 using a 6° position of the standard observer. Color was measured in six predetermined point at the surface of eight tortillas of each type.

Texture Analysis

Rupture force (N) and rupture distance (mm) were measured using a one-dimensional extensibility test as

described in Bejosano et al.¹⁷ with a TA.XT2 Texture Analyzer equipped with a 25-kg load cell (Stable Micro Systems, Goldalming, UK). Test speed was adjusted to 1.0 mm/s. Duplicated analysis was carried out on 12 tortillas for each type.

Total Antioxidant Capacity

The total antioxidant capacity (TAC) values were determined as previously described in Pellegrini et al.¹⁸ by means of the Trolox-equivalent antioxidant capacity (TEAC) method. The method is based on the ability of antioxidant molecules to quench the long-lived 2,2'-azinobis-3-ethylbenzthiazoline-6-sulfonate radical cation (ABTS⁺), a blue-green chromophore with characteristic absorption at 734 nm, compared with that of Trolox, a water-soluble vitamin E analogue. The prototypes were analyzed in triplicate, and results were reported as millimoles per kilogram of Trolox equivalents (means±standard deviation).

Glycemic Index Determination

Servings of tortillas containing 50 g of available carbohydrate were fed to subjects to evaluate the GI as described by Wolever et al.¹⁹ Subjects consumed either the tortillas, served with 500 ml of water, or the glucose solution standard (twice) over a 12–15-min period. Finger-prick capillary blood samples were taken prior to the meal and at six fixed time points after the first bite. Blood glucose was determined using a glucose analyzer with a combined enzymatic–electrochemical system (YSI 2300 STAT PLUS, Yellow Spring Instruments, Ohio, USA).

Statistical Analysis

All data are expressed as means and standard deviations with the exception of GI values (mean±SEM). SPSS (Version 13.0, SPSS, Chicago, IL, USA) statistical software was used to perform one-way analysis of variance and Tukey's honest significant difference test at a 95% confidence level ($p \leq 0.05$) to identify differences among groups.

Results

Tortilla Production, Acceptability, and Physical Characterization

Tortilla prototypes were developed using a standard wheat tortilla as the reference and modifying its formulation in an effort to include ingredients that are known to have a positive impact on the nutritional quality of the product.

Tortillas macronutrients composition was determined, and the results were summarized in Table 2. SOY and CSK samples resulted to be significantly higher in proteins, lipids, fiber, and ashes and lower in carbohydrates and water as compared to the STD. KAM and CAR compositions were more similar to that of the STD with the exception of higher fiber content in KAM and slightly higher carbohydrates and lower moisture content in CAR.

Tortillas were analyzed for sensory acceptability, and the results are reported in Table 3. The lowest score corresponded to the most preferred tortilla for the corresponding attribute. CSK resulted as the most preferred prototype for all the parameters evaluated (texture, color, flavor, and overall acceptability), while the other prototypes were statistically equally acceptable (for all attributes) to a consumers panel.

Tortilla STD had brightness (L^*)=80±1, redness (a^*)=2±1, and yellowness (b^*)=19±1 (Table 4) reflecting literature data,²⁰ and it was not distinguishable from SOY. Carrot juice enhanced redness and yellowness (a^* =6±1, b^* =34±3, CAR) because of the presence of carotenoids. The use of whole kamut flour (KAM) resulted in a brighter product (L^* =84±1). CSK had the lowest brightness (L^* =74±1) because of a more cooked appearance possibly related to occurrence of more extensive Maillard's reaction during cooking; redness (a^* =5±1) and yellowness (b^* =27±1) of CSK also increased due to the presence of carotenoids from the carrot juice.

Textural properties were measured by a one-dimensional extensibility test and indicated that STD and KAM were softer products (rupture force of 2.5±0.1 and 2.5±0.4 N in STD and KAM, respectively, Fig. 1a). CAR, SOY, and more significantly, CSK were harder with force at rupture of 3.5±0.2, 3.8±0.5, and 4.5±0.1 N, respectively. The

Table 2 Tortilla prototypes composition (standard deviation is given in parenthesis)

	STD	CAR	SOY	KAM	CSK
Proteins (%)	9.4 (0.2)	9.6 (0.2)	15.8 (0.3)	9.5 (0.2)	15.4 (0.3)
Carbohydrates (%)	48.6	49.8	35.26	44.3	32.7
Lipids (%)	7.4 (0.1)	8.0 (0.2)	15.1 (0.3)	7.6 (0.2)	15.1 (0.3)
Fiber (%)	3.5 (0.1)	3.3 (0.1)	7.2 (0.2)	6.7 (0.2)	10.1 (0.3)
Water (%)	28.8 (0.4)	26.9 (0.3)	23.6 (0.3)	29.4 (0.4)	23.4 (0.3)
Ashes (%)	2.3 (0.1)	2.4 (0.1)	3.1 (0.1)	2.5 (0.1)	3.4 (0.1)

Table 3 Sensory analysis results of tortilla prototypes

Parameter	STD	CAR	SOY	KAM	CSK
Texture	59 ^a	61 ^a	57 ^a	53 ^a	40 ^b
Color	64 ^a	50 ^a	52 ^a	61 ^a	43 ^b
Flavor	58 ^a	60 ^a	55 ^a	57 ^a	40 ^b
Overall acceptability	53 ^a	61 ^a	65 ^a	51 ^a	40 ^b

Same letters within each row do not significantly differ ($p \leq 0.05$)

changes in formulation also affected the extensibility of the products (Fig. 1b): Rupture distance of STD was 7.6 ± 0.7 mm, comparable to SOY (8.3 ± 1.3 mm) and CSK (7.7 ± 0.5 mm). The use of carrot juice and, more significantly, of wholemeal kamut flour increased products' extensibility to 10.0 ± 0.8 (CAR) and 13.3 ± 1.4 mm (KAM) indicating a higher flexibility and resistance to handling of these products. Textural changes may possibly be related to the competition (at a molecular–microstructural level) of the different solids (gluten, starch, sugars, proteins, fiber, etc.) for water resulting in different degrees of hydration and plasticization of the polymers in the different matrices. It is well documented that small molecules (e.g., sugars, salt, polyols), fiber, and soy significantly affect water state and distribution in complex matrices and, consequently, the textural properties of food products.^{21–28}

Tortilla Nutritional Characterization

The TAC of each product, as valued by the TEAC method, is shown in Fig. 2. KAM and CSK prototypes had the significantly higher TAC values (4.18 ± 0.18 and 4.03 ± 0.11 mmol/kg) than SOY (2.92 ± 0.24 mmol/kg). The TAC values of STD (3.45 ± 0.56 mmol/kg) and CAR (3.32 ± 0.23 mmol/kg) were intermediate.

CSK was also characterized by the lowest GI (43 ± 6), as shown in Fig. 3. This tortilla had a GI significantly lower than all the other samples (STD, 68 ± 5 ; CAR, 78 ± 4 ; KAM, 63 ± 7) with the only exception of SOY (51 ± 4).

Discussion

Tortillas with enhanced nutritional value were developed incorporating ingredients with well-documented nutritional

Table 4 Color attributes of tortilla prototypes

	STD	CAR	SOY	KAM	CSK
L^*	80 ± 1^b	80 ± 1^b	80 ± 1^b	84 ± 1^a	74 ± 1^c
a^*	2 ± 1^c	6 ± 1^a	2 ± 1^c	2 ± 1^d	5 ± 1^b
b^*	19 ± 1^c	34 ± 3^a	19 ± 1^c	19 ± 1^c	27 ± 1^b

Same letters within each column do not significantly differ ($p \leq 0.05$)

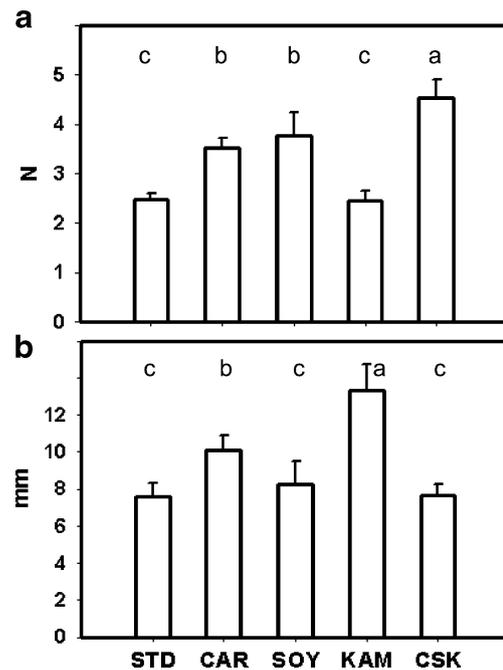


Fig. 1 Textural attributes of tortilla prototypes: force at rupture (a) and rupture distance (b). Bars with the same letter do not significantly differ ($p \leq 0.05$)

functionality into a standard wheat tortilla formulation. In this respect, carrot juice was used to partially substitute the water of the recipe in an attempt to increase the carotenoids content (α - and β -carotene, in particular) and, consequently, increase the total antioxidant content of the product. The maximum amount of carrot juice that could be added to maintain a good quality product was $\sim 17\%$ (Table 1).

Soy and, more specifically, some of its components called isoflavones have raised the interest of nutritional research in recent years. Recent experimental and epidemiological studies have provided convincing evidence for a

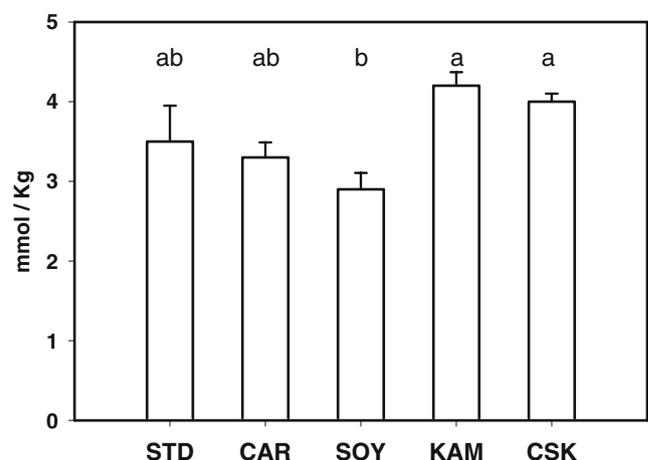


Fig. 2 TAC of tortilla prototypes. Bars with the same letter do not significantly differ ($p \leq 0.05$)

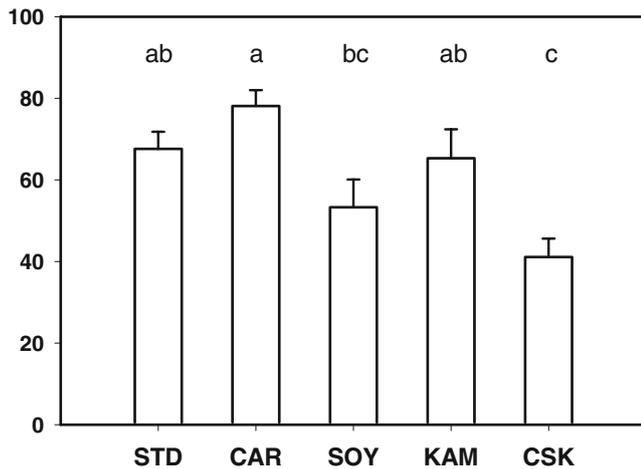


Fig. 3 GI of tortilla prototypes. Bars with the same letter do not significantly differ ($p \leq 0.05$)

variety of health benefits derived from the consumption of soy and soy food products. For example, based on the recent literature, both animal and human studies demonstrate that phytoestrogenic soy isoflavones favorably impact bone health.²⁹ Moreover, soy protein is characterized by a very good nutritional value (amino acid composition and digestibility³⁰) and may lower low-density lipoprotein cholesterol when it replaces dairy protein or a mixture of animal proteins.³¹ Soy is known to have a negative effect on the development and acceptability of bakery products (e.g. alter gluten network formation, beany flavor^{32–35}), and, therefore, its use in substitution (~28%) to wheat flour was accompanied by a larger amount of margarine (8%) and addition of wheat gluten (1.6%; Table 1) to obtain a pliable and high-quality product.

Kamut is an ancient type of wheat rich in protein (by between 15% and 40%), minerals such as magnesium and zinc, vitamins B and E, and unsaturated fatty acids.³⁶ Whole kamut flour was substituted to wheat flour to increase the dietary fiber content as high fiber intakes are associated with lower serum cholesterol concentrations, lower risk of coronary heart disease, reduced blood pressure, enhanced weight control, better glycemic control, reduced risk of certain forms of cancer, and improved gastrointestinal function.³⁷

An attempt to create a product encompassing all the nutritional properties considered was successfully carried out with the development of the tortilla CSK. This product contained simultaneously carrot juice, whole soy flour, and whole kamut flour, and its production required the modifications from the standard recipe described for the tortillas with one nutritional benefit (e.g., wheat gluten addition, reduction in water quantity, increased carrot juice, and increased margarine content; Table 1).

The simultaneous combination of carrot juice, soy, and wholemeal kamut resulted in a very interesting product

(CSK) that was not only the most acceptable by the consumers but also showed the lowest GI and was relatively high in TAC, overcome only by the kamut-based one. Regarding these two variables that have been considered for the evaluation of nutritional quality, TAC considers the cumulative action of all the antioxidants present in foods and beverages, thus providing an integrated parameter rather than the simple sum of measurable antioxidants. It has been previously demonstrated that dietary TAC is inversely and independently correlated with biomarkers of systemic inflammation, and this could be one of the mechanisms explaining the protective effects against cardiovascular diseases of antioxidant-rich foods such as fruits, whole cereals, and red wine.³⁸ It was also previously observed that dietary TAC is an independent predictor of plasma β -carotene and that the inverse relationship observed between plasma β -carotene and risk of chronic diseases associated to high levels of oxidative stress (i.e., diabetes and cardiovascular disease), as well as the failure of β -carotene supplements alone in reducing such risk, could be related to this feature.³⁹

Regarding GI, high GI diets are associated with high-grade liver steatosis, particularly in insulin-resistant subjects,⁴⁰ and high intake of rapidly absorbed carbohydrate appears to play an important role in the risk of breast cancer in Mexican women.⁴¹ Moreover, apart from the already cited benefits on metabolic variables, low GI diets have been often related to better cognitive and physical performances.^{8,42} The mechanisms through which the GI of food items can be reduced are various and related to several technological and compositional aspects. In this study, probably, the two main variables influencing GI are the fiber and fat content of the products. However, discriminating which factors are providing the actual benefit goes beyond the scope of this work, which, like many others dealing with this nutritional characteristic, is observational and not designed with the aim of producing low GI foods.

In conclusion, we have developed and characterized a highly acceptable tortilla with a high nutritional value for what regards the most recent observation about the effects of low GI food items and high intake of dietary antioxidants. The uniqueness of our work is to have considered these two nutritional features at once, thus creating a food product able to positively affect health with two separate but strongly linked mechanisms, only very recently observed in the scientific literature.

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