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Substitution of wheat flour with cauliflower flour in bakery products: effects on chemical, physical, antioxidant properties and sensory analyses

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Abstract

Cauliflower is considered as a rich source of dietary fiber and it possesses both antioxidant and anticarcinogenic properties. Disposal of the non-edible portion of cauliflower, remains a crucial problem, otherwise, if used properly, these wastes may serve as good sources of nutrients for produce different foods. The objective of the study was using cauliflower stalk flour (CSF) and cauliflower leaves flour (CLF) flours in making biscuits. Biscuits were formulated with 3 different concentrations of CSF. It was analyzed chemical composition, pH, titratable acidity and oxalate. The total phenolic content was measured by Folin-Ciocalteu method and antioxidant activity was conducted by DPPH assay with five different extractors. The leaf and stalk had a productivity flour of 8,45% (CSF) and 4,0% (CLF), respectively. Both flours contained a high amount of crude fiber (47,07% and 31,13%, respectively). Better antioxidant activity was found in CLF than in CSF. The biscuits prepared would be classified, as products ready for consumption with high fiber content. All attributes acceptability index reached above 70%. The biscuits prepared can be considered products' quality and acceptance, especially since these products do not yet exist in the market.

Introduction

Brassica vegetables include some economically interesting crops such as cabbage, broccoli, cauliflower, and turnip, which have consumed the entire world. A high consumption of these vegetables is associated with a decreased risk of cardiovascular diseases, cancer and degenerative pathologies. Compared to other vegetables, cauliflower has higher antioxidant potential which makes them very interesting crops from the consumer's point of view. It is considered as a rich source of dietary fiber and it possesses both antioxidant and anticarcinogenic properties (Podsedek, 2007).

Cauliflower has the highest waste index, i.e. ratio of non-edible to edible portion after harvesting, and thus generates a large amount of organic solid waste, which creates a foul odor on decomposition. Disposal of the non-edible portion of cauliflower (cauliflower waste), which contributes to about 45–60% of the total weight of the vegetable, remains a crucial problem (Oberoi *et al.*, 2007).

Fruits and vegetables are extensively processed for the beverage manufacture generating a large amount of residue which is frequently discarded, causing disposal problems. Classically, the outer layers and extremities of fruits and vegetables are removed during processing, mainly by peeling and pressing; they comprise essentially stalks, peels, seeds and crashed pulp which still contain large amounts of bioactive molecules and biopolymers, resulting in a considerable nutritional loss (Ayala-Zavala *et al.*, 2010; Sousa *et al.*, 2011; Ferreira *et al.*, 2013). There is very scanty literature available on the use of vegetable residues, especially cauliflower waste, for food production (Oberoi *et al.*, 2008).

Cereals, legumes, vegetables, fruits, lentils, nuts and seeds are found to be rich sources of dietary fibre in residues and it is also associated with antioxidant compounds such as polyphenols and there is a growing interest in the beneficial properties of dietary fibre associated with polyphenolic antioxidants (Vitaglione *et al.*, 2008; Navarro-Gonza´ lez *et al.*, 2011)

The consumption of polyphenol-rich foods or beverages seems to be associated with the prevention of some types of diseases (Llorach *et al.*, 2005). The medicinal value of plants have assumed a more important dimension in the past decades owing largely to the discovery that their extracts contain not only minerals but also a diverse array of secondary metabolites with antioxidant potentials (Ahenkora *et al.*, 1998; Akinmoladun *et al.*, 2007). These antioxidants have been implicated in the therapeutic

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effects of several plants and vegetables that are used in traditional medicine (Marthur and Marthur, 2001; Kumar *et al*., 2005)

In the present study, the substitution of wheat flour for cauliflower stalk flour (CSF) and cauliflower leaf flour (CLF) in bakery products and their influence on nutrient and fiber content, antioxidant properties and sensory analyses have been studied with the aim of exploiting the potential value of the dietary fibre and antioxidant activity from cauliflower flour.

Material and Methods

Material and flour preparation

Fresh white cauliflower (*Brassica oleracea* L. var. botrytis) variety was obtained in local distributor in Rio de Janeiro. Each whole cauliflower was separated at different four parts as follows: florets (edible portion), upper stem, stalks and leaf midribs (non-edible portion) and the remaining of the plant (lower stem and leaves) and then weighed. Stalks and leaf samples were washed with subsequent drying in a ventilated oven at 40°C for 16h for leafs and 70°C for 12h for stalks till moisture of 7-8% to obtained cauliflower stalk flour (CSF) and cauliflower leaf flour (CLF). Wheat flour and other ingredients used in sweet biscuits were obtained from the local markets.

Formulation of biscuits

Preliminary studies (data not shown) have demonstrated the non-viability of CLF used for producing biscuits with little acceptable sensory characteristics. Biscuits were made with 3 different concentrations of CSF (Table I). The formulation of biscuits was obtained by fitting the original formulation described by Perez (2002).

The percentage of vegetable cream was increased relative to CSF, by facilitating the function of connecting mass. All dry ingredients were mixed together first, then the vegetable cream and soy lecithin, to form mass, was added water gradually to the point of connecting to open the mass. The biscuits were ordered directly in rectangular pans, and baked in an oven preheated to 150ºC for 15 to 20 min, or until fully baked.

Physical characteristics of biscuits

For physical evaluations, the biscuits were crushed in the mill. Samples of each formulation (BCSI, BCSII and BCSIII) were evaluated for productivity. The analyzes were conducted with biscuits from the same batch sampled randomly in triplicate. The characterization was realized when the biscuits reach ambient temperature. The productivity was calculated by the ratio of the weight of biscuit post baking and pre baking expressed as a percentage. The specific volume (VE) of biscuits was determined by the displacement of the mass occupied (millet seed) and determined its volume in a graduated cylinder.

Nutrient content

Cauliflower flours (CSF and CLF) and biscuits (BCSI, BCSII and BCSIII) were analyzed for proximate composition (moisture, protein, fat and ash) by the standard procedures of the Association of Analytical Chemists (AOAC, 2000). The content of total dietary fiber was first analyzed using an enzymatic gravimetric method (Asp *et al*., 1983). Total carbohydrates were calculated by difference. The calories were estimated using the conversion factors ATWATER: 4 kcal / g for protein, 4 kcal / g for carbohydrate and 9 kcal / g for lipids. Were performed pH, total titrable acidity described by AOAC (2000). The oxalate content was described by the second Iwuoha and Kalu (1995).

Extraction of samples

The samples of flour and biscuits were extracted with 5 different solutions extractors: methanol (I), methanol 50% (II), methanol acidified (III), methanol 50%: acetone 70% (IV) and acetone 70% (V). It were weighed 0.5 g of sample and diluted in 10ml of extracting solution for 1 hour under stirring protected from light. The extracts were filtered and completed in 100 ml with distilled water.

Total phenolic assay

Total phenolic content of samples was determined according to the Folin Ciocalteu method as described by Singleton (1999) with slight modifications. In the test were used 0.5 ml of the extracts (0.1, 1.0 and 1.5 $mg.ml^{-1}$), 2.5 ml of Folin-Ciocalteau and 2.0 ml of sodium carbonate 4%. The reading was performed after 2 hours spectrophotometer at 750 nm. Gallic acid in the specific concentration range (0-100 $mg.mL^{-1}$) was used to construct a calibration curve. The concentration of total phenolic compounds in the samples is expressed as gallic acid equivalents, which reflect the phenolic content as the amount of gallic acid in mg per gram dry weight of the samples. Total phenolic compounds in the samples were analyzed in 3 replications, and the results were averaged.

DPPH assay

We used the same test extracts phenolics. Was performed by capture DPPH radical (2,2-diphenyl-1 pricril-hydrazyl). For analysis of the samples was added 3 mL of methanol solution of DPPH, a 0.5

mL aliquot of samples (0.1, 1.0 and 1.5 mg.ml^{-1}) containing different extracts. The readings were taken on a spectrophotometer at 517 nm, 30 minutes after the start of the reaction. All determinations were performed in triplicate and include a control (no antioxidant). The decline the reading of optical density of the samples was correlated with the control, establishing the percentage of DPPH discoloration. Allowing calculate, after the establishment of the equilibrium of the reaction, the amount of antioxidant spent to reduce DPPH 50% (EC50 value) (Brand-Williams *et al*., 1995).

Consumers evaluation

The biscuits were analyzed by means of sensory testing acceptance/preference in relation to the attributes color, aroma, flavor and texture. The biscuits were evaluated by 105 consumers randomly selected, divided into 5 blocks of 21 consumers of different age groups and gender, students and employees of University. The samples were provided to consumers in a tray of acrylic with three samples coded with three-digit numbers in balanced complete block, a plug with the test, and a term of informed consent for participation in research. Mineral water was supplied at room temperature to the palate cleansing between samples. The plug of the test contemplated identification data, information on frequency of consumption of biscuits, acceptance test, preference and purchase intent.

In acceptance test consumers classified in relation to the attributes color, aroma, taste and texture using a hedonic scale of 9 points (9 = like extremely, 5 = indifferent, 1 = extremely dislike) for the three samples. In the preference test the tasters identified which of the three samples was preferred. In test purchase intent, consumers responded on a scale of certainly buy a certainly would not buy the biscuit chosen.

Statistical analyzes

The data on the composition and sensory acceptance were calculated by analysis of variance (ANOVA) and Tukey's test for comparison of means at a significance level of 5% using the GraphPad Prism 5.0.

Results and Discussion

Productivity and chemical composition of cauliflower stalk and leaf flours

The productivity of stalk and leaf was as follows: starting from 22 kg of cauliflower was obtained 22.5% and 27.7% of fresh leaf and stalk respectively. The leaf and stalk had a productivity flour of 8.45% and 4.0% respectively. Mauro *et al.* (2010) found a productivity of 5.4% flour stalk of kale and 3.8% flour stalk spinach. The productivity of flour was reduced due to the high moisture content of fresh leaf and stalk: 91.52% to 96.01% and the leaf to the stalks.

The mean results of the energy and chemical composition of CLF are moisture (6.91 ± 0.51), ash (7.65 ± 0.11), protein (26.60 ± 0.61), fiber (33.22 ± 1.35), fat (4.09 ± 0.15), carbohydrate (27.11 ± 0.58) and calories (267.73 ± 0.45). CSF showed moisture (5.4 ± 0.84), ash (9.75 ± 0.16), protein (8.93 ± 0.76), fiber (51.72 ± 2.25), fat (2.14 ± 0.74), carbohydrate (22.74 ± 2.49) and calories (160.86 ± 1.11).

CLF and CSF showed moisture within the maximum required by industry to 15% for flour and bran (Brazil, 2005) with low water activity. The percentage of moisture is similar to other farinaceous integrals (Lima, 2007; Mauro *et al*., 2010). It was observed that both flours contained a high amount of crude fiber, which was found to be at 31.13 and 47.07%, respectively for CLF and CSF. Lipid contents were found in small amounts, which were represented about 4.09% in CLF and 2.14 in CSF. CLF had higher amounts of both protein and carbohydrate contents than CSF, on contrast, CSF had a higher percent of ash. Thus, the white cauliflower flours are considered a good source of protein and fiber and can therefore be used in the production of novel foods, as mentioned by Oberoi *et al.* (2008) and Abul Fadl (2012).

Compared with the composition of carrot leaf flour studied by Pereira *et al.* (2003), the flours analyzed had higher protein (15.15%) and fiber content (12.01%) and lower percentage of carbohydrates (52.65%). Thereupon, cauliflower flours are considered a good source of protein, minerals and fiber. Therefore it should be utilized in food fortification. These data are in accordance with those obtained by Oberoi *et al*. (2008), Stojceska *et al.* (2008) and Thilagam *et al. (*2011). An increase of fiber content in cauliflower flour may contribute to health. It has been reported that intake more fiber resulted in increasing faecal bulk and show the ability to bind a number of substances, including cholesterol and gastric juice (Jenkins *et al*., 1998; Jiménez-Escrig and Sánchez-Muniz, 2000).

Phenolic compounds and antioxidant activity of cauliflower leaf (CLF) and stalk (CSF) flours

CLF was significantly higher when compared to total phenolic compounds than CSF, regardless extraction solution used (Figure 1). The highest

Table 1. Formulation of biscuits with cauliflower stalk flour (CSF)

	Formulation			
Ingredients (g/%)	**Control**	**BCSI**	**BCSII**	**BCSIII**
Wheat Flour	100	90,00	85,00	80,00
Cauliflower stalk flour	0	10,00	15,00	20,00
Vegetal cream	14,80	14,80	16,80	18,80
Salt	2,20	2,20	2,20	2,20
Sodium Bicarbonate	0,12	0,12	0,12	0,12
Condiments	6,80	6,80	6,84	6,88
Water	60,00	60,00	60,00	60,00

Table 2. DPPH reduction (%) and radical scavenging activities (IC50) of cauliflower leaf flour (CLF) and stalk flour (CSF) by different extracting solutions (I-V)

		% DPPH REDUCTION				
		$0.1 mg.ml^{-1}$	$1.0 mg.ml^{-1}$	$1.51 mg.ml^{-1}$	R^2	IC_{50}
CLF	I	5.03 ± 1.02^a	29.38 ± 2.12^a	43.51 ± 3.25^a	0.997	3.25
	II	4.80 ± 0.26^a	25.97 ± 1.45^a	49.84 ± 4.17^a	0.998	3.03
	III	35.88 ± 3.20^b	80.19 ± 7.25^b	87.01 ± 2.36^b	0.848	1.31
	IV	8.44 ± 1.25^c	74.19 ± 4.37^b	89.12 ± 5.89^b	0.883	1.82
	V	11.85 ± 0.23^d	71.43 ± 3.98^b	80.68 ± 4.93^b	0.848	1.86
CSF	I	5.52 ± 0.63^a	32.47 ± 2.54^a	41.23 ± 7.25^a	0.920	3.32
	II	7.14 ± 0.32^b	28.08 ± 1.99^a	34.42 ± 5.21^a	0.913	3.96
	III	45.94 ± 2.96^c	71.92 ± 4.28^b	75.32 ± 4.32^b	0.835	1.02
	IV	6.49 ± 1.05^b	29.87 ± 3.06^a	37.34 ± 2.58^a	0.919	3.65
	V	4.38 ± 0.89^a	23.38 ± 5.01^a	29.55 ± 3.79^c	0.920	4.46

Mean values within a column and group with different superscript letters differ significantly ($p < 0.05$)

amount of phenolic compounds was identified using the extractor IV (methanol 50%: acetone 70%) for CLF and extractor I (methanol) for CSF with average values in mg of gallic acid/g of 9.57 ± 0,20 (CLF) and 1.84 ± 0,20 (CSF).

Flavonoids and glucosinolates (GLS) found in cauliflower have been the focus of much research, due to their potential as health promoting phytochemicals. Phenolic compounds and GLS exhibit antioxidant and antimicrobial properties and have been investigated extensively regarding their ability to lower the risk of cardiovascular diseases and cancer (Volden *et al.*, 2009).

The antioxidant effects of extracts of various of wheat flour (WF), lupine flour (LF) and their blends at different concentration (5,10 and 15 %) were measured by Ahmed (2014). It was observed scavenging activity of 3.31 and 20.62% in wheat and lupine flours, respectively. In other sudy, Babbar *et al*. (2011) concluded that all methanolic extracts showed considerable difference in antioxidant activity. Scavenging activity of 83% for kinnow seed and 77% for litchi seed and grape seed was observed in the extracts.

Our results revealed high differences in DPPH reduction comparing CLF and CSF extracts were shown in reducing power assay (Table 1). Better antioxidant activity was found in CLF than in CSF flours indicated by lower IC_{50} values. Table 2 shows an average decrease in the IC_{50} of DPPH reduction of

Table 3. Chemical composition (%) and energy value of BCSI, BCSII, BCSIII

Components	BCSI	BCSII	BCSIII
Moisture	7.64 ± 0.11^a	6.63 ± 0.18^{ab}	6.23 ± 0.89^b
Ash	4.19 ± 0.02^a	4.61 ± 0.02^a	5.02 ± 0.01^a
Protein	$11,82 \pm 0,34^a$	$10,54 \pm 0,57^a$	$11,12 \pm 0,73^a$
Fat	10.06 ± 0.13^a	11.33 ± 0.45^b	12.43 ± 0.18^c
Fiber	6.38 ± 1.26^a	10.69 ± 2.99^{ab}	13.53 ± 1.94^b
Carbohydrate	60.3 ± 1.42^a	57.1 ± 2.23^a	52.6 ± 2.73^a
Calories (Kcal)	394.53 ± 0.75^a	387.45 ± 3.72^a	383.08 ± 0.29^a

Mean values within a line with different superscript letters differ significantly ($p < 0.05$, tukey´s test)

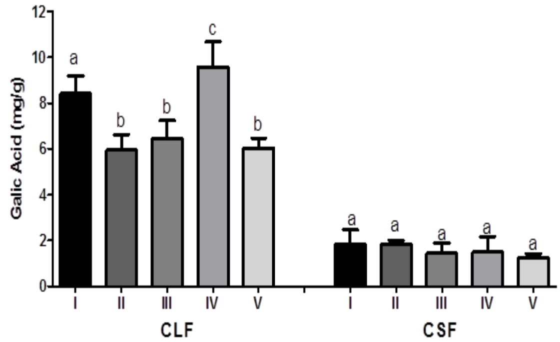

Figure 1. Total phenolic compounds (mg gallic acid/g) by different extracting solutions (I-V) in CLF and CSF. Means with different letter differ significantly ($p<0.05$, Tukey´s test)

CLF (1.05±0.58) when compared to CSF (1.75±0.91) regardless of the extraction solution used, indicating a strong antioxidant activity. The maximum reduction observed in DPPH was 89.12 ± 5.89 % for CLF (extractor IV) and 75.32 ± 4.32 for CSF (extractor III) (Table 3).

The investigated extracts differed significantly ($p<0.05$) in their total phenolic content that is contributed to the different abilities to to exhibited differences in antioxidant activities (% DPPH reduction). Zielinski and Kozlowska (2000) have the statistically significant correlation between antioxidative activities and total phenolics of cereals and their fractions. A correlation between antioxidative activity and rutin content or total flavonoids content in buckwheat cultivars has been shown (Jiang *et al*., 2007). In our work, high content of phenolic compounds was not reflected in an increase of antioxidant activity.

Chemical characteristics and composition of biscuits

The mean results of the chemical composition of the biscuits are shown in Table 3. Compared the chemical composition of biscuits Table 3 shows that the percentage of moisture biscuits decreases as the concentration increases the CSF, which can be explained by the percentage of water used in the formulations also be reduced as the concentration is

Table 4. Means for attributes of acceptability of products prepared from CSF

Atributtes	BCSI	BCSII	BCSIII
Color	6.86 ± 1.63^{a}	6.51 ± 1.58^{ab}	6.31 ± 1.68^{b}
Aroma	6.40 ± 1.34^{a}	6.30 ± 1.36^{a}	6.12 ± 1.59^{a}
Texture	6.57 ± 1.62^{a}	6.19 ± 1.60^{a}	6.35 ± 1.80^{a}
Flavor	6.83 ± 1.28^{a}	6.31 ± 1.67^{a}	6.39 ± 1.88^{a}

Means followed by the same letter horizontally do not differ p> 0.05

Table 5. Index of acceptability (%) of products prepared from CSF

Atributtes	BCSI	BCSII	BCSIII
Color	76.2^{a}	72.3^{a}	70.1^{a}
Aroma	71.1^{a}	70.0^{a}	68.0^{a}
Texture	73.0^{a}	68.7^{a}	70.5^{a}
Flavor	75.8^{a}	70.1^{a}	71.0^{a}

Means followed by the same letter horizontally do not differ p> 0.05

increased because the amount of water is calculated according to the quantity of wheat flour. Other studies using mixed flours showed a small increase in moisture as you increase the fiber content (Mauro, 2010; Perez, 2002). Although the increasing levels of ash and carbohydrates decrease the growth of CSF in the formulations, the ash content, protein and carbohydrates showed no significant difference (p <0.05) between the formulations analyzed.

The lipid content was increased in accordance with the different formulations, however, may be due to the need for increasing amounts of vegetable cream, in order to impart sufficient plasticity to the mass, so that it can be handled properly. This need to increase the amount of fat may be related to increasing the dietary fiber content in mass caused by the addition of flour stalk, but still, the values were similar to those found in the study by Mauro (2010) with cookies using stems of spinach and kale. The dietary fiber content also increased with increasing proportions of flour stalk, which is attributed to the high content of total dietary fiber found in the CSF (47.07 g/100 g). The biscuits prepared would be classified, as products ready for consumption with higher fiber content (more than 6 g fibers/100 g). The caloric value of biscuits did not differ significantly (p <0.05) between the samples. This value is still significantly lower than that found by other researchers in studies of cookies and crackers (Perez, 2002).

Physical characteristics of biscuits

The specific volume is of great importance in determining the quality because it is generally influenced by the quality of the ingredients used in the formulation (Perez and Germani, 2007). We also emphasize that the values of the specific volume obtained with biscuits made with different concentrations of flour mixed CSF are similar to the specific volume (1.55 cm^3) of cream cracker biscuit type found by VITTI et al. and specific volume (1.44 cm^3) cracker eggplant flour prepared by Perez and Germani (2007).

Weights and specific volume of biscuits pre- and pos-baking does not differ statistically, demonstrating a high degree of homogeneity in biscuits produced. The levels of pH and titratable acidity was significant difference between the samples, increasing as the concentration of CSF. Santana *et al*. (2011) found approximate values of pH and acidity on your biscuit made with passion fruit peel flour when compared with biscuits formulated with the CSF, 5.50 and 5.23 respectively.

Antioxidant activity of biscuits

The percentage of DPPH radical scavenging activity significantly increased by 77.74 % for the biscuit substituted with cauliflower flour compared to control (100% wheat flour). In addition, the biscuit substituted with cauliflower flour had lower IC values than control product. Analyzing the IC_{50} of different extracts of the different biscuits, it is found that the antioxidant activity is shown in BCSIII when extracted with methanol (I). This fact is justified to the biscuit the largest concentration be used flour stalk of cauliflower that has a strong antioxidant activity.

The most effective solution in the extraction of phenolic compounds was the solution III (acidified methanol). Fialho *et al*. (2010) evaluated phenolic compounds and antioxidant capacity of organic and conventional vegetables, which used a solution of 1.2 M HCl and 50% methanol, as the extraction solution to extract the phenolic hydrolysates. The work concluded the ability of HCl to extract fraction of phenolic compounds bound and quantifies the free and bound phenolics, generating probably increased in phenolic content and antioxidant activity, observed in our biscuits produced.

Consumers evaluation

Table 4 shows the means of the scores assigned by the hedonic scale of four attributes evaluated experimental biscuits: color, aroma, texture and flavor. In general, can say that the testers gave notes between 6 (like slightly) and 7 (like regular) for all attributes in all the biscuits. The main criteria for acceptance of foods enriched with dietary fiber are: good performance in processing, good stability and appearance, and satisfaction in aroma, color, texture and feeling left by the food in the mouth (Thebaudin *et al*., 1997). And according to the analysis of the attributes in question, biscuits meets these criteria.

Table 5 represents the index of acceptability biscuits.Ac cording to Teixeira *et al*. (1987) for a product to be accepted in terms of their sensory properties, it is necessary to obtain an index of acceptability of at least 70%. All attributes (color, aroma, texture and flavor) acceptability index reached above 70%, with the exception of texture and flavor in BCSII and BCSIII but did not differ significantly ($p < 0.05$) between samples. According Fasolin *et al.* (2007), other studies conducted with different types of biscuits have demonstrated strong tendency of industries and researchers to promote the enrichment of cookies because, being a low-cost can easily be consumed by the less privileged social classes.

Conclusion

The production of biscuits with flour stem cauliflower proved entirely feasible with regard to the acceptability of the product. Biscuits produced from CSF showed good acceptability and high fiber content. Cookies prepared can be considered product quality and acceptance, especially since these products are not yet fully established in the world.

This study reported a feasible and sustainable food processing without waste generation. Results showed that the high fiber, protein and mineral contents and also the WHC and OHC of the FVR flour are potentially suitable for use in food applications as a new low-calorie and functional raw material. The designed products presented high fibre content, reasonable consumer acceptance and were microbiologically stable. This research promotes the reducing food waste since whole plant tissues have been used leading to the maximum exploitation of food raw materials.

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