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The Effect of Different Drying Processes on Physicochemical Characteristics and Antioxidant Activity of *Brassica* spp. Cultivars from Northern Atlantic Portugal

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The main objective of this study was to evaluate the effect of two different drying methods on physicochemical characteristics and antioxidant activity of different *Brassica* spp. cultivars in order to produce a dietary flour. Fresh cabbages were subjected to two drying methods: convective air-drying (80 °C, during 2h with the previous blanching) and freeze-drying. An increase in crude protein, carbohydrates and fibre was observed. Regarding protein and fibre content, there is a tendency to be higher with convective air-drying method. There were significant changes in all analysed colour parameters of dried samples. “Coração de Boi” flours, obtained with both drying methods, revealed the lowest differences compared to a fresh sample. Results from both drying methods showed the positive effect that dehydration had on antioxidant activity. Flours produced with convective air-drying with previous blanching, resulted in a decrease of EC_{50} values (EC_{50}= 0.74 - 1.64 mg/mL). Although, the values of EC_{50} were still lower in the case of lyophilized samples and the lowest values were found for “Penca da Póvoa” and “Galega” flours (EC_{50}= 0.47 - 0.48 mg/mL). From these results, it was concluded that both drying processes have advantages on physicochemical characteristics and antioxidant activity of the products. *Brassica* flours, from both drying methods, presented an antioxidant potential and therefore may constitute an important natural source of antioxidants to be applied to different food matrices, especially flours of “Galega” and “Penca da Póvoa”, from freeze-drying method, which showed the lowest EC_{50} values.

1. Introduction

Brassica vegetables, including cabbages, represent one of the major vegetable crops grown, worldwide, constituting an important part of a well-balanced diet (Ferreres et al., 2017). They feature large biodiversity, in which landraces and primitive cultivars still play a major role in the cultivation systems. They are traditionally cultivated and widely consumed in rural communities in Northern Portugal and are a traditional component of Southern European Atlantic Diet (SEAD) (Vaz-Velho et al., 2016). From the sustainability and food security point of view, it is also considered to have great potential due to the use of processing cabbage by-products and surplus, which are usually discarded, to produce value-added products (Tanongkankit et al., 2012). Cabbage is consumed either raw or processed in different ways, especially boiled and it is included as one of the main principal ingredients of soups, playing an important role Portuguese gastronomy. However, the high moisture content (approximately 86% wet basis (w. b.)) compromises its preservation, with quality losses occurring immediately after harvest and consequently cannot be preserved for more than a few days under ambient conditions (Oliveira et al., 2015). Several studies reported that the consumption of these vegetables is associated to the preventive effect against some chronic diseases, such as cancer, atherosclerosis, diabetes (Taveira et al., 2009). These beneficial effects in *Brassica* vegetables have been attributed to the presence of bioactive compounds with antioxidant activity such as phenolic, carotenoids and flavonoids compounds (Aires et al., 2011). The content of natural antioxidants among *Brassica* vegetables varies

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significantly between and within their subspecies because of different maturity at harvest and conditions of growth, soil state and postharvest storage (Podsedek et al., 2006). Interest in the role of free radical scavenging-antioxidants in human health has prompted research in the fields of horticultural and food science to assess the antioxidant phytochemical in fruits and vegetables (Rokayya et al., 2013). Drying of vegetables has been practiced in many countries to increase the shelf life of products allowing to preserve and store dried products for months or even years without nutrient losses. However, dehydration is unavoidably accompanied by physical, biological and chemical modifications, which may affect the overall quality of the dried products and consumer`s acceptance. Numerous processing techniques have been used for drying fruits or vegetable, such as hot air-drying or freeze-drying. Hot air-drying offers dehydrated products that can have an extended life shelf-life of a year, but unfortunately with a drastically reduced quality from that of the original foodstuff. Prior to the air-drying process, pre-treatment with chemicals or blanching can be used to inhibit various undesirable enzymatic reactions improve the final quality of the products (Fellows, 2009). Freeze-drying is a gentle dehydration technique, representing the ideal process for the production of high-value products and is well known for its ability to maintain product quality (Karam et al., 2016). The main objective of this study was to evaluate the effects of both two different drying methods on physicochemical characteristics and antioxidant activity of different *Brassica* spp. cultivars in order to produce a dietary flour.

2. Material and Methods

2.1 Sample Preparation

Samples of "Penca da Póvoa" (*Brassica oleracea* L. var. *costata*), "Galega" (*Brassica oleracea* L. var. *acephala* DC), "Portuguesa" (*Brassica oleracea* var. *acephala*) and "Coração de Boi" (*Brassica oleracea* L. var. *capitata*) cabbages were obtained by a company from Póvoa de Varzim (UPN, Lda.) from northern Portugal and maintained at 4 °C until the moment of its use, approximately 24h. Before each experiment, fresh samples were disinfected with 44% active chlorine (Glow Professional), washed with tap water, dried with absorbent paper and were then sliced into smaller pieces (Julienne sliced). To inhibit enzymatic browning reactions a blanching step was coupled to convective drying. The sliced cabbages were blanched in hot water 95 °C ± 2 °C for 2 minutes. After blanching, samples were immediately cooled in cold water (4 °C). Since freeze-drying is a process that maintains the sensorial characteristics of fresh product, blanching was not performed.

2.2 Drying experiments

Fresh cabbages were subjected to two different drying methods: convective air-drying and freeze-drying. Drying by forced convection with hot air (5 m/s) was carried out with a convectional electric oven (Fagor VPE-061, Spain). The fresh cabbage sample, with previous blanching, was spread evenly on a perforated tray and drying experiment was carried out at 80 °C, during 2h with air velocity set to 5 m/s. In freeze-drying, samples with any pre-treatment, were previously frozen at -80 ± 2 °C (SANYO, Japan) and then placed in the freeze drier (ALPHA 1-2 LD plus, CHRIST, Germany) for 48 hours. The dehydrated cabbages by convective air-drying were then cooled at room temperature. Finally, all dried samples were crushed to flour texture, and finally, packed and sealed in polyethylene bags until further analysis. For each cabbage, drying experiments were carried out in triplicate.

2.3 Chemical composition

Proximate composition, both for fresh sample and obtained flours, including moisture content, ash, crude protein, carbohydrates, and crude fibre were first analysed. The moisture content was determined by a gravimetric method at 103 °C (AOAC 925.10). Ash content was determined by incineration at 550 °C (AOAC 935.39-B). Crude protein content was determined by estimating the nitrogen content using the Kjeldahl method (AOAC 935.39-C) and multiplying by the factor 6.25. Carbohydrates were determined by 3,5-dinitrosalicylic acid colorimetric method (DNS) (James, 1995). DNS forms a red-brown reduction product, 3-amino-5-nitrosalicylic acid, when heated in the presence of reducing sugar. The intensity of the colour developed at 540 nm may be used to determine the available carbohydrate content of the food following hydrolysis of carbohydrates to reducing sugars. Crude fibre was determined by the ceramic fibre filter method (AOAC 962.09). Samples were analysed for the above composition in triplicate.

2.4 Colour Measurement

Colour changes of fresh and dried samples was assessed using a Minolta CR300 (Konica Minolta, Japan) and the colour system CIE L*, a*, b*. Lightness (*L* *), redness to greenness (*a**) and yellowness to blueness (*b**) were measured, and the total colour difference was calculated ($\Delta E^a = \sqrt{(L)^2 + (a)^2 + (b)^2}$; $L = L - L_0$, $a = a - a_0$

and $b = b - b_0$. Each sample was measured six times and the result was presented as an average ± standard deviation.

2.5 Antioxidant Activity

2.5.1 Extraction

A total of 5 g of lyophilized cabbage was extracted with 15 mL of 80% methanol and the mixture was filtered. Then, 15 mL of 80% methanol were added to the residue and the mixture was filtered again. The last step was repeated one more time. The filtrate was centrifuged at 2000 g for 20 minutes, and the supernatant was completed to 50 mL with 80% methanol (extract stock solution). Dilutions of 1:2 and 1:5 were performed.

2.5.2 Determination

The antioxidant activity of each sample was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method based on Brand-Williams et al. (1995). A volume of 0.3 mL of extract stock solution and diluted extracts (in methanol) was mixed with 2,7 mL of DPPH (60 μM) in methanol and let to stand at room temperature, in the dark, for 30 minutes prior to measuring the solution at 515 nm using the spectrophotometer (Varian Cary 50, California). The control was a DPPH solution containing absolute methanol instead of the sample. The antioxidant activity was based on the measurement of the reducing ability of fresh and dried cabbages extracts towards the radical DPPH. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation: $\%RSA = [(A_{DPPH} - A_S) / A_{DPPH}] \times 100$, where A_s is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH dilution. The extract concentration providing 50 % of radicals scavenging activity (EC_{50}) was calculated from the graph of % RSA against the concentration of the extract.

2.6 Statistical Analysis

The data were analysed and presented as mean values with standard deviations. SPSS (IBM, USA version 25) was used for statistical analyses. The analysis of variance (ANOVA) and Tukey test were used to determine statistically different values at a significant level of p<0.05.

3. Results and Discussion

3.1 Proximate composition of fresh samples and respective flours

The proximate composition of fresh cabbages and respective flours (expressed on dry weight basis) are shown in Table 1. Moisture content of fresh cabbages was 80 - 95 % (w/w) and after both drying methods, moisture fall to 3.5 – 13 % (w/w).

Table 1: Proximate composition of fresh sample and respective flours

		Composition (g/100g dry mass)				
	Drying Method	Moisture	Ash	Protein	Carbohydrates	Fibre
Penca da Póvoa	Fresh	80.3 ± 0.12^a	1.86 ± 0.14^c	7.71 ± 0.36^c	16.7 ± 1.14^b	12.8 ± 0.29^c
	Convective air-drying	12.6 ± 0.11^b	7.18 ± 0.08^b	16.7 ± 0.34^a	27.9 ± 4.50^a	15.4 ± 0.83^b
	Freeze-drying	5.37 ± 0.20^c	10.0 ± 0.03^a	13.8 ± 0.05^b	26.5 ± 4.20^a	16.8 ± 0.59^a
Galega	Fresh	80.4 ± 0.23^a	0.62 ± 0.01^c	19.4 ± 0.88^c	15.4 ± 1.81^b	11.0 ± 0.37^c
	Convective air-drying	6.56 ± 0.22^c	9.51 ± 0.09^b	25.4 ± 0.92^a	20.4 ± 1.19^a	13.3 ± 0.11^a
	Freeze-drying	7.34 ± 0.18^b	11.9 ± 0.12^a	21.0 ± 1.87^b	21.9 ± 0.58^a	12.5 ± 0.19^b
Portuguesa	Fresh	89.7 ± 0.09^a	0.78 ± 0.23^b	9.27 ± 0.34^c	19.4 ± 1.27^c	14.8 ± 0.23^c
	Convective air-drying	9.73 ± 0.06^b	10.7 ± 0.24^a	19.5 ± 0.47^b	25.7 ± 0.70^a	15.1 ± 0.07^a
	Freeze-drying	8.14 ± 0.22^c	11.2 ± 0.74^a	15.2 ± 1.24^a	22.5 ± 1.92^b	11.2 ± 0.14^b
Coração de Boi	Fresh	93.6 ± 0.16^c	0.99 ± 0.12^c	6.78 ± 2.18^a	26.3 ± 6.3^c	5.30 ± 0.22^c
	Convective air-drying	3.44 ± 0.32^a	7.57 ± 0.31^a	15.8 ± 0.95^a	39.7 ± 0.93^b	12.1 ± 0.11^a
	Freeze-drying	8.64 ± 0.27^b	6.25 ± 0.08^b	15.4 ± 3.21^a	49.6 ± 0.17^a	9.54 ± 0.09^b

Same letters in the same column and cabbage indicate that values are not significantly different (p>0.05)

As can be seen, drying methods did have a significant effect on the analysed parameters in all cabbages. Regarding protein content there is a tendency to be higher with convective air-drying method (16.7 - 25.4 %). The same behaviour was observed for fibre content, in almost all flours (12.1 – 15.4%), except for “Penca da Póvoa” flour (16.8%). This may be due to previous blanching and the consequent loss of minerals, vitamins and sugars to the balancing water, resulting in changes of total solids, increasing protein and lipid content on a dry basis (Nilnakara et al., 2009). On the other hand, protein content was lower in freeze-dried product, although it is probable that the quality of nutrients is maintained, since is a gentle dehydration technique representing the ideal process for the production of high-value dried products (Karam et al., 2016).

3.2 Colour Properties

The colour parameters (L*, a*, b*) and the total colour differences (ΔE) of fresh and dried cabbages by both drying methods are shown in Table 2.

Table2: Colour parameters of L, a* and b* obtained for fresh cabbages and respective flours*

	Drying Method	*L**	*a**	*b**	Δ*E*[a]
Penca da Póvoa	Fresh	38.9 ± 2.40^{b}	-13.5 ± 1.75^{c}	22.2 ± 3.69^{a}	
	Convective air-drying	40.6 ± 2.18^{b}	-2.07 ± 0.35^{a}	11.5 ± 1.57^{c}	16.3 ± 2.91^{b}
	Freeze-drying	47.6 ± 0.75^{a}	-6.77 ± 0.51^{b}	12.6 ± 1.09^{b}	14.7 ± 2.30^{a}
Galega	Fresh	27.1 ± 2.92^{c}	-8.68 ± 0.91^{c}	13.5 ± 1.89^{a}	
	Convective air-drying	33.6 ± 0.54^{b}	-1.33 ± 0.15^{a}	5.97 ± 0.41^{b}	12.8 ± 1.83^{b}
	Freeze-drying	44.4 ± 0.85^{a}	-7.51 ± 0.34^{b}	12.9 ± 0.65^{a}	17.4 ± 3.11^{a}
Portuguesa	Fresh	25.7 ± 2.99^{c}	-5.74 ± 2.02^{b}	10.2 ± 1.05^{b}	
	Convective air-drying	37.6 ± 1.78^{b}	-1.49 ± 0.37^{a}	9.03 ± 1.29^{b}	13.1 ± 2.00^{b}
	Freeze-drying	44.7 ± 0.26^{a}	-7.32 ± 0.17^{b}	13.2 ± 0.15^{a}	19.5 ± 2.77^{a}
Coração de Boi	Fresh	54.3 ± 2.09^{a}	-5.11 ± 0.80^{b}	20.4 ± 2.05^{a}	
	Convective air-drying	44.3 ± 2.08^{c}	-7.95 ± 0.69^{c}	15.8 ± 0.45^{b}	11.47 ± 0.83^{a}
	Freeze-drying	47.1 ± 0.46^{b}	-0.66 ± 0.23^{a}	12.0 ± 0.20^{c}	11.96 ± 3.16^{a}

Same letters in the same column and cabbage indicate that values are not significantly different (p>0.05)

There were significant changes in all analysed parameters of dried samples when compared to fresh ones. It can be observed that samples subjected to drying methods became slightly lighter than the fresh product, except “Coração de Boi” flour. “Penca da Póvoa”, “Galega” and “Portuguesa” samples subjected to convective air-drying with previous blanching showed less changes in lightness. Regarding “Coração de Boi” flour, the lightness of freeze-dryied sample was similar to the fresh sample. The greenness was also affected. Flours of “Penca da Póvoa” and “Galega” lost their greenness in both methods. Those losses were more evident with air-drying method with previous blanching. These results are not backed up by literature that reported blanching causes removal of intracellular air, leading to protection of colour pigments (Tanongkankit et. al, 2012). On the other hand, freeze-drying method seems to be able to preserve the original green colour (Karam et al., 2016). “Portuguesa” flour also lost its green colour with air-drying method but with freeze-drying became even greener. However, convective air-drying, with previous blanching, had an exactly opposite effect in “Coração de Boi” flour leading to an even greener sample, with slightly loss of yellowness, corroborating the results Nilnakara et al. (2009). In all dried samples, except for “Portuguese” lyophilized flour, slight loss their intensity of yellow occurred. Regarding ΔE values, “Coração de Boi” flours, obtained with both drying methods, revealed the lowest differences compared to fresh sample.

3.3 Antioxidant Activity

The antioxidant activity of fresh and respective flours under two dehydrating methods is represented in Table 3.

Table 3: Antioxidant activity of fresh cabbages and respective flours

	Drying Method	RSA (%)	EC_{50}(mg/mL)
Penca da Póvoa	Fresh	48.0±1.73^a	9.81±0.32^c
	Convective air-drying	60.9±4.73^b	0.93±0.05^b
	Freeze-drying	79.9±0.81^c	0.47±0.00^a
Galega	Fresh	38.1±2.19^a	12.1±0.79^c
	Convective air-drying	43.2±1.50^b	1.19±0.04^b
	Freeze-drying	70.0±4.61^c	0.48±0.02^a
Portuguesa	Fresh	20.0±2.88^a	23.4±3.06^c
	Convective air-drying	32.2±0.81^b	1.64±0.03^b
	Freeze-drying	45.8±0.13^c	1.13±0.01^a
Coração de Boi	Fresh	42.7±2.54^a	8.49±0.52^c
	Convective air-drying	47.8±3.23^b	0.74±0.05^b
	Freeze-drying	62.4±0.07^c	0.58±0.00^a

Same letters in the same column and cabbage indicate that values are not significantly different ($p>0.05$)

From the results it becomes apparent that both drying methods have a considerable positive effect on the antioxidant activity. Flours produced in this study, with convective air-drying at 80 °C, during 2h, with previous blanching, resulted in a decrease in EC_{50} values, which means that antioxidant activity was higher than in fresh samples. In literature, several studies reported that antioxidant activity was positively correlated with drying temperatures (Vega-Gálvez et al., 2009). According with Nicoli (1997), natural antioxidants are lost during heating and the overall antioxidants properties of some foods could be maintained or enhanced by the development of new antioxidants. In contrast, Oliveira (2015) reports that there is a negative correlation between the antioxidant activity and the drying temperature in “Galega” cabbage without blanching. Therefore, it is possible that blanching offers protection to the sample and is advantageous to the increase of antioxidant activity. According to Korus (2011), in spite of losses in antioxidant content caused by blanching at the preliminary stage of processing kale samples, dried blanched leaves resulted in lower antioxidant loss. The values of EC_{50} were still lower in the case of lyophilized samples and the lowest values were found for “Penca da Póvoa” and “Galega” flours (EC_{50}= 0.47 - 0.48 mg/mL). According to Marchi (2015), convectional drying process causes a significant loss of the antioxidant capacity of pomegranate peel extracts compared with freeze-drying. This may occur due to the fact that freeze-drying samples are not exposed to heat, and, consequently, oxidation is diminished (Nindo et al., 2003). In addition, the freeze-drying low processing temperature and the virtual absence of air oxygen during process minimizes degradation reactions (Karam et al., 2016). Moreover, the loss of bioactive compounds was further found to be negligible in freeze-dried sample (Asamis et al., 2003).

4. Conclusion

The present work studied the effect of physicochemical characteristics and antioxidant activity of fresh cabbages and cabbage flours after drying process by convective air-drying (with previous blanching) and freeze-drying. With the results obtained, it was possible to conclude that both drying methods have advantages on physicochemical characteristics of cabbage flours when compared to the fresh samples. Regarding colour properties (ΔE), for both drying methods, “Coração de Boi” flour showed the lowest coulor differences. Concerning antioxidant activity, it becomes apparent that both drying methods have a considerable positive effect on cabbage flours. Lyophilized samples of “Penca da Póvoa” and “Galega” flours showed the lowest values of EC_{50} (EC_{50}= 0.47 - 0.48 mg/mL). As a general conclusion, *Brassica* flours, for both drying methods, presented an antioxidant potential and therefore may constitute an important natural source of antioxidants to be applied to different food matrices, especially flours of “Galega” and “Penca da Póvoa”, from the freeze-drying method, which showed the lowest EC_{50} values.

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