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SHORT COMMUNICATION

Antioxidant capacity, polyphenolics and pigments of broccoli-cheese powder blends

Krishan Datt Sharma · Kathrin Stähler · Bronwen Smith · Laurie Melton

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Abstract Novel products were developed by combining freeze dried and conventionally dried broccoli sprouts powder with the commercially available cheese powder. Quality assessment of the blended products during the development process and of the final product was done by sensory analysis and estimation of total antioxidant capacity, total polyphenolics, total chlorophyll and total carotenoids. Freeze drying was more efficient in preserving the original nutritional and sensory characteristics of the dried broccoli sprouts. Blending of the samples including the blend with a least weight proportion of broccoli sprouts powder showed an improved content of healthy compounds. Based on chemical data and sensory analysis, it was concluded that the blended cheese powder containing up to 20% freeze dried broccoli powder was an acceptable product.

Keywords Broccoli · Cheese · Antioxidant · Polyphenol · Carotenoid · Chlorophyll

Many studies correlate a reduction by 50% in the risk of developing some forms of cancer with an intake of 400–600 g of fruit and vegetables per day. The single isolated components did not have the same effect on health as the whole fruit or vegetable itself because their bioactivity is lost when consumed in the isolated form (Heber 2004). Phytochemicals are supposed to limit the expression of oxidative stress in the body because of their biochemical ability to reduce the free radicals (Chu et al. 2002). The other benefits of including fruit and vegetables in the diet include glucosinolates (Heber 2004) or dietary fibre (Fahey 2003) and the connected lower BMI (Striegel-Moore et al. 2006). Cheese has health promoting characteristics associated with certain immune functions in the body. However, it lacks vitamin C, polyphenolics and short of carotenoids which play important role as antioxidants in the human body.

Broccoli (*Brassica oleracea* var *italica*) belongs to the family *Cruciferae* and over 100 hybrid cultivars of *Brassica* commercially exist. All plants of the genus *Brassica* like broccoli, Brussels sprouts, cabbage, cauliflower, collard greens and kohlrabi contain glucosinolates. Glucosinolates are known to have fungicidal, bactericidal and cancer protective characteristics (Fahey 2003). Further, Fahey (2003) has reported that the broccoli is one of the richest sources of health promoting glucosinolates, antioxidants and essential nutrients like total dietary fibre (3.0 g/100 g), calcium (48 mg/100 g), magnesium (25 mg/100 g), selenium (3.0 μg/100 g), zinc (0.4 mg/100 g), ascorbic acid (93.2 mg/100 g), folate (71 μg/100 g) and β-carotene (779 μg/100 g). Thus broccoli has high amount of antioxidants such as vitamin C, Zn, Se and β-carotene. Carotenoids are the major group of vitamins present in broccoli followed by vitamin E and biotin (Souci et al. 2000). Besides, broccoli contains a recognisable amount of flavonoids which are associated with decreasing lipid oxidation, arthritis, cancer and cardiovascular disease. Chu et al. (2002) showed that broccoli is a vegetable with the highest amount of total phenolic content as compared to other commonly eaten vegetables like carrot, spinach, onion or cucumber.

All the vegetables contain various amounts of anti-nutritional factors like phytic acids, tannic acid and/or oxalic acid and blanching is an effective method to

K. D. Sharma (✉)
Department of Food Science & Technology, Parmar University, Solan 173 230, India
e-mail: krishansharmakd@rediffmail.com

K. Stähler · B. Smith · L. Melton
Food Science Programme, University of Auckland, Auckland, New Zealand

substantially reduce these factors (Mosha et al. 1995). Despite, the fact that the boiling of broccoli for more than 10 min greatly reduces its benefits (Anon 2009) but, a significant decrease in oxalic acid and nitrate as anti-nutritional factors in leaves of broccoli on cooking has been reported by Santos (2006). Mrkic et al. (2006) have reported that high-temperature short-time processes maximise the antioxidant activity of broccoli and this activity is positively and significantly correlated with the free polyphenol content but not to the total polyphenol and kaempferol.

Cheese is a good source of minerals like Ca, Mg, P and fat-soluble vitamins (Henning et al. 2006) and also has anti-caries functions (Buttris 2003). Besides, cheese has functional ingredients like bioactive peptides or conjugated linoleic acid. Conjugated linoleic acid is associated with anti-cancer, anti-diabetic and anti-obesity potentials (Henning et al. 2006). Cheese contains a small amount of phenols (O'Connell and Fox 2001) but lacks vitamin C and many important antioxidants (Buttris 2003). Therefore, the main aim of this study was to develop a novel product by blending dried broccoli sprouts powder and cheese powder for better nutrition and health promoting principles like antioxidants and other beneficial phytochemicals.

Dehydration of broccoli Fresh broccoli sprouts (moisture content 89.8%) were procured from a local market. Before drying, the sprouts were treated with heat to inactivate chlorophyllase and polyphenoloxidase. The sprouts were cut into pieces of about 2 cm diameter, blanched for 15 s in hot water at 98 °C with a broccoli–water ratio of 1:4 with slight modification in the blanching time as recommended by Maharaj and Sankat (1996). To produce a broccoli powder the blanched sprouts were dried in freeze dehydrator and conventional dehydrator. Freeze drying is defined as a process during which water is removed by freezing the product and then converting the ice directly into vapour without passing the liquid state (Mellor and Bell 2003). Freeze drying was carried out at −49° to −50 °C at a vacuum of 43 MT using Vitris Sentry™ 12SL freeze dryer (Virtis, Gardiner, New York, USA). The conventional drying process was performed at 70 °C in a vacuum dryer (Vakuumtrockenschrank VT6025, Heraeus, Germany). Dried sprouts from both freeze and conventional dehydration process having moisture content of 4.9 and 5.1%, respectively were ground in household mixer to develop a fine powder.

Fine powder prepared from broccoli sprouts in the product development laboratory of the Food Science discipline of the University of Auckland, Auckland, New Zealand was blended with the cheese powder [Blue cheese powder of Great American Spice Company containing 18 g fat, 24.7 g carbohydrates, 43 g proteins, 100 mg cholesterol and 1,910 mg sodium per 100 g of powder] purchased from a local market. There were 7 blends developed by mixing broccoli–cheese in different proportions viz., Product I (3:97), Product II (5:95), Product III (10:90), Product IV (15:85), Product V (20:80), Product VI (30:70) and Product VII (50:50). Consumer's acceptance of the developed blended products were tested on a 9-point Hedonic scale by presenting the samples to a panel comprising of nine semi-trained members. Each of the seven samples of the blended broccoli–cheese powder products were without reconstitution with water presented to a panel of judges and four questions related to the colour, flavour, texture and overall acceptability were asked. These questions were supposed to be answered in relation to the dry cheese powder (control). The scores ranged from 1 (dislike extremely), 5 (neither like nor dislike) and 9 (like extremely).

Total antioxidant capacity (TAC) was measured by Lako et al. (2006) method and using the ferric reducing antioxidant power (FRAP) assay according to the procedure described by Benzie and Strain (1999). FRAP assay measures the ferric reducing ability of the sample in terms of reducing capability of the formed ferric-tripyridyltriazine complex. The absorbance was measured for 4 min every 15 s in a Spectrophotometer (UV mine 1240, UV–VIS spectrophotometer, Shimadzu, Japan) at 593 nm and the TAC was calculated by using the following formula:

$$\frac{0-\text{to } 4-\text{min } \Delta \text{ A593 nm test sample}}{0-\text{to } 4-\text{min } \Delta \text{ A593 nm standard}} \times 2(\mu\text{g/ml})$$

Total polyphenols (TPP) were measured using the Folin-Ciocalteu method described by Sellappan and Akoh (2002). The absorbance was measured in a spectrophotometer at 765 nm and the TPP concentration was calculated with a standard curve of the gallic acid standards. The amount of chlorophyll was determined because the green colour of broccoli is mainly due to the amount of chlorophyll present in the sprouts which could affect the consumer's acceptance of the developed blends. The total carotenoids content was also measured. The method described by Smith (2006) was followed and the amount of chlorophyll and total carotenoids was calculated with the following formula:

$$\text{Chlorophyll A}(\text{C}_\text{A})(\mu\text{g/ml}) = 11.24\text{A}_{662} - 2.04\text{A}_{645}$$

$$\text{Chlorophyll B}(\text{C}_\text{B})(\mu\text{g/ml}) = 20.13\text{A}_{645} - 4.19\text{A}_{662}$$

$$\text{Total carotenoids}(\mu\text{g/ml}) = (1000\text{A}_{470} - 1.9\text{C}_\text{A} - 63.14\text{C}_\text{B})/214$$

All chemical data were analyzed by mean and standard deviation of 3 independent samples. In addition, the chemical results and the results of the sensory evaluation were analysed by analysis of variance (Johnson and Bhattacharyya 2001).

Total antioxidant capacity (TAC) Data in Table 1 show that the TAC in fresh broccoli sprouts is about 6–7 times higher than that of the dried broccoli. However, freeze dried broccoli had a higher TAC compared to conventional dried powder. An increase of TAC in the cheese blends containing an increasing amount of both freeze and conventional dried broccoli has been noticed. While the TAC in freeze dried sample containing 50% broccoli powder exceeded the activity of antioxidants of the plain dried sample, the TAC in conventional dried samples stayed below the amount present in the plain powder. During drying reducing sugars such as glucose can react with amino acids catalysed by heat and form Maillard products. The comparison between freeze and conventional dried broccoli powder shows that freeze dried samples had higher capacity of total antioxidants. This is due to the quality saving characteristics of freeze drying (Mellor and Bell 2003).

Total polyphenols (TPP) Fresh broccoli sprouts contained more polyphenols as compared to other dried samples (Table 1). The freeze dried sample without blending showed about 25% decrease of the TPP content to that of fresh sprouts. On the other hand, conventional dried broccoli without blending showed a significant decrease in the total amount of polyphenols as compared to fresh and freeze dried broccoli. There was a linear increase of the TPP content in the blended samples containing 3–15% freeze dried broccoli sprouts powder. However, the TPP content decreased slightly in samples containing 20% and 30% of freeze dried broccoli sprouts powder. The sample containing 50% freeze dried broccoli sprouts powder contained more polyphenols as compared to the sample containing 30%. The results of the conventional dried broccoli sprouts powder blended with cheese cannot be described as a linear increase in polyphenols. Here the sample containing 10% conventional dried broccoli sprouts powder seems to have the lowest amount of TPP while the sample containing 50% powder had the highest amount, followed by 5, 20, 15, 30, 3 and 10%. Statistical analysis showed that samples containing 5–10%, 15–20% and 30–50% freeze dried broccoli sprouts powder, are not significantly different. But, all blended samples contained more TPP as compared to the plain conventional cheese powder. In Folin-Ciocalteu method the determination of TPP is non-specific i.e. all phenolic groups which are present in the sample extract are detected. This includes the phenolic compounds which are bound to proteins on the condition that these proteins are extractable and present in the extract (Shahidi and Naczk 2005). Although, the protein binding affinity of phenols is known, the structure of protein-polyphenol complexes

Table 1 Total antioxidant capacity (TAC), total polyphenols (TPP), total chlorophyll and total carotenoids of broccoli, cheese and their blends

Sample	TAC, AAE µg/g dwb	TPP, GAE µg/g dwb	Total chlorophyll, µg/g dwb	Total carotenoids, µg/g dwb
Fresh broccoli	372.8	2,525.0	1,446.6	248.4
FD broccoli	66.6	1,959.2	258.8	190.3
CD broccoli	53.7	389.2	157.6	56.6
Cheese powder	12.6	1,298.5	9.6	5.0
FDBCPB (3:97)	15.4	3,389.9	41.2	15.9
CDBCPB (3:97)	9.7[a]	1,176.4[a]	17.1	11.7[a]
FDBCPB (5:95)	25.4	3,866.9[a]	55.6	21.2
CDBCPB (5:95)	15.4[a]	1,458.8[b]	35.8	14.3[a; b]
FDBCPB (10:90)	45.9[a]	3,950.6[a]	90.1	35.6
CDBCPB (10:90)	29.8	1,120.2[a]	71.7	14.6[b]
FDBCPB (15:85)	47.5[a; b]	4,589.5[c]	140.9	44.3
CDBCPB (15:85)	38.5[b]	1,203.2[a]	105.3	22.2
FDBCPB (20:80)	53.5[b]	4,504.2[c]	147.1	59.8
CDBCPB (20:80)	43.2[b]	1,352.1	115.8	26.1
FDBCPB (30:70)	65.2	4,146.8[b]	169.4	104.3
CDBCPB (30:70)	44.8[b]	1,183.7[a]	126.0	36.6
FDBCPB (50:50)	74.5	4,239.1[b]	187.0	140.4
CDBCPB (50:50)	56.3	1,490.9[b]	138.9	40.5
LSD for FDBCPB	6.1	101.6	5.4	3.2
LSD for CDBCPB	8.4	87.7	4.1	2.8

Mean value of 3 replications; Letters a, b, c as superscripts on the mean values in columns indicate statistical equal treatments of the blends in respective category

FD freeze dried; *CD* conventional dried, *FDBCPB* freeze dried broccoli–cheese powder blend; *CDBCPB* conventional dried broccoli–cheese powder blend; *AAE* ascorbic acid equivalent; *dwb* dry weight basis; *TPP* total polyphenols; *GAE* gallic acid equivalent; *LSD* least significant difference

($p \leq 0.05$), ($n=3$)

remains to be investigated (Papadopoulou and Frazier 2004).

Total chlorophyll Freeze dried broccoli sprouts powder (258.8 μg/g) and conventional dried broccoli sprouts powder (157.6 μg/g) contained less chlorophyll than that of fresh broccoli sprouts (1,446.6 μg/g) on dry weight basis (Table 1) indicating that drying destroyed the chlorophyll significantly. However, some of the chlorophyll which was entrapped in the chloroplast in fresh broccoli might have been freed during the blanching and drying process. But, apparently this did not affect the loss during the drying process. The content of chlorophyll in freeze dried broccoli sprouts is about one third higher as compared to the conventional dried broccoli indicating that the freeze drying preserves phytochemicals more efficiently than the conventional drying method. The amount of chlorophyll increased nearly linear (Table 1) as the amount of broccoli powder increased in different blends in both freeze and conventional dried broccoli powders. However, the content of chlorophyll in freeze dried powder was much higher as compared to conventional dried cheese powder. The chlorophyll in conventional cheese sample (9.6 μg/g) was due to the method of analysis chosen in this study. The maximum absorbance for chlorophyll ranges from 645 to 662 nm (Rodriguez-Amaya 2003). The results of the blended cheese samples showed an increase in the chlorophyll content as they contained an increasing amount of broccoli powder and none of the readings were statistically non-significant. All blended samples contained more chlorophyll than that of the plain cheese powder thus affecting the colour ratings. Concurrently, more chlorophyll in the blended samples attributed towards nutritional value since it contains magnesium as a central atom which is able to function as an antioxidant.

Total carotenoids Data in Table 1 revealed that the amount of carotenoids in fresh broccoli is higher as compared to both (freeze and conventional) dried broccoli samples. While the amount of carotenoids in the freeze dried powder was about 58 μg/g less as compared to fresh broccoli sprouts, but the amount of carotenoids in conventional dried samples was about 5 times less than fresh broccoli sprouts. Table 1 also shows that the carotenoids in the blended freeze dried samples increase nearly exponentially to that of the blends with conventional dried broccoli increasing slightly linear. The results of the total carotenoids present in the samples are almost similar to that of the chlorophyll content. There was threefold increase of the carotenoids in the freeze dried samples as compared to the conventional dried samples. In addition, the amount of carotenoids in both dried samples is lower when compared with the fresh broccoli sprouts. However, the amount of carotenoids obtained in this study for fresh broccoli is nearly threefold higher as compared to the reported values (Souci et al. 2000) and this could be explained by the fact that only broccoli sprouts which have a higher nutritional density as compared to the stem were used for this study. However, Bernhardt and Schlich (2006) showed that the bioavailability of carotenoids increases during cooking and also during blanching processes. This is because cooking and blanching disrupts the molecular linkage between carotenoids and protein in the food matrix. Hence, it can be assumed that the availability of carotenoids can also be enhanced in the extract after a blanching and heat treatment. This would explain the minimum loss in the plain freeze dried powder as compared to fresh broccoli. The results of the blended samples (Table 1) showed a linear relationship between the seven blends. Cheese powder in this study contains 5 times more carotenoids as compared to listed values for Parmesan cheese (Souci et al. 2000). Statistically, there were non-significant similarities between the freeze dried blends in terms of the total carotenoids. The content of carotenoids was higher even in the 3% broccoli powder containing sample as compared to plain cheese.

Sensory quality Statistical analysis showed that different blends can be grouped according to the least significant difference ($p \leq 0.05$). In terms of colour, the blends containing 3–15% freeze dried broccoli were statistically equal and had the highest score (Table 2). On the other hand, the blend containing 3–10% showed the best flavour characteristics with the best rating for the blend containing 10% broccoli powder. The results for the texture and mouth feel of the product exhibited that all samples were statistically at par except the blend with 50% broccoli. The overall

Table 2 Sensory score of freeze dried broccoli–cheese powder blends

Sample	Sensory attribute*			
	Colour	Flavour	Texture	OAA
3% FDBP+97% CP blend	7.4^{a}	$6.8^{a;\ b;\ c}$	6.33^{a}	6.2^{a}
5% FDBP+95% CP blend	7.3^{a}	$7.1^{a;\ b}$	6.6^{a}	6.8^{a}
10% FDBP+90% CP blend	$7.2^{a;\ b}$	7.2^{a}	6.3^{a}	6.3^{a}
15% FDBP+85% CP blend	$6.7^{a;\ b}$	$5.7^{b;\ c;\ d}$	$5.6^{a;\ b}$	6.0^{a}
20% FDBP+80% CP blend	$5.8^{b;\ c}$	$5.6^{c;\ d}$	$5.7^{a;\ b}$	5.6^{a}
30% FDBP+70% CP blend	$4.4^{c;\ d}$	$4.8^{d;\ e}$	$5.0^{a;\ b}$	3.4^{b}
50% FDBP+50% CP blend	3.1^{d}	3.4^{e}	4.3^{b}	3.2^{b}
LSD ($p \leq 0.05$)	1.5	1.5	1.6	1.5

*Mean value of 9 observations; *LSD* least significant difference; Letters a, b, c, d, e as superscripts on the mean values in columns indicate statistical equal treatments of the blends in respective category

FDBP freeze dried broccoli powder; *CP* cheese powder; *OAA* overall acceptability

acceptability score for sensory evaluation indicated that the blended samples containing up to 20% broccoli powder were well accepted because they were statistically not significant despite the fact that the sample containing 20% broccoli powder had 15 times more chlorophyll as compared to plain cheese powder.

From the present study, it was concluded that the blended cheese powders containing 3–15% freeze dried broccoli powder were excellent products. If only flavour is to be considered, the blend containing 10% freeze dried broccoli sprouts powder was the product of choice on hedonic scale. Sensory overall acceptability score indicated that up to 20% broccoli powder can be incorporated into the cheese powder. Therefore, the blending of blue cheese powder with 20% freeze dried broccoli powder is recommended for the manufacture of a novel type of product for health conscious consumers.

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