

## Effect of black and red cabbage on plasma carotenoid levels, lipid profile and oxidized low density lipoprotein





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#### 1. Introduction

Functional foods that provide benefits beyond their traditional nutritional value have attracted much interest. In fact several studies have shown that diet plays a protective role against the development of human diseases (diabetes, obesity,

### ABSTRACT

The bioavailability of carotenoids and the protective effect exerted by Brassica vegetables against lipoprotein peroxidation has not previously been investigated in humans. The aim of this study was to evaluate the bioavailability of carotenoids in black (Brassica oleracea L. var. acephala subvar. Laciniata L) and red cabbage (Brassica oleracea L. var. capitata L.f. rubra) and their protective effect against LDL oxidation. Moreover, we studied the effect on plasma lipid profile. Thirty-eight healthy volunteers (23 females and 15 males) participated in the 2-week intervention study which included a daily portion (300 g) of black and red cabbage. Plasma lutein and  $\beta$ -carotene levels and total antioxidant capacity after dietary intervention were significantly increased. The results obtained demonstrated that Brassica supplementation favourably influenced serum lipid profile with a significant decrease in total cholesterol, LDLcholesterol and oxidized LDL. Moreover, our results strengthen the hypothesis that the effect of dietary intervention could be modulated by baseline conditions of the subjects.

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cardiovascular disease and metabolic syndrome) (Baboota et al., 2013; Liu, 2013; Magrone et al., 2013; Scicchitano et al., 2014). Vegetables as functional foods include Brassica vegetables notably Brassicaceae (e.g., cruciferous family such as broccoli, cabbage, cauliflower, and Brussels sprouts). In fact they exert a protective effect against many chronic degenerative diseases, including cancer (Herr & Buchler, 2010; Manchali, Murthy,

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Abbreviations: BMI, body mass index; CVD, cardiovascular disease; FOX assay, ferrous oxidation-xylenol orange assay; HDL, high density lipoproteins; HDL-C, HDL-cholesterol; LDL, low density lipoprotein; LDL-C, LDL-cholesterol; ORAC, oxygen radical absorbance capacity; ox-LDL, oxidized LDL; TC, total cholesterol; TG, triacylglycerols

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& Patil, 2012). Glucosinolates are particularly abundant in Brassica vegetables and are believed to be bioactive compounds responsible for many of the biological effects attributed to them (Herr & Buchler, 2010; Yeh & Yen, 2009). However, these vegetables are an important source of other essential compounds such as polyphenols, carotenoids and phytosterols that exert an anti-inflammatory and antioxidant effect (Bjorkman et al., 2011; Fernández-León, Fernández-León, Lozano, Ayuso, & González-Gómez, 2012; Kopsell, Kopsell, Lefsrud, Curran-Celentano, & Dukach, 2004; Podsedek, 2007).

The biological effect of extracts of Brassica oleracea L. var. acephala DC. (kale) B has previously been studied in vitro (Kural, Kucuk, Yucesan, & Orem, 2011) and in animal studies (Kim et al., 2009; Lemos, Santin, Klein, Niero, & de Andrade, 2011; Melega et al., 2013). Moreover, the effect of 3-month kale (Brassica oleracea acephala) juice supplementation on coronary artery disease risk factors has been studied in hypercholesterolaemic men (Kim, Yoon, Kwon, Park, & Lee-Kim, 2008).

However, the bioavailability of bioactive nutrients and the protective effect exerted by Brassica vegetables against lipid peroxidation of low density lipoproteins (LDL) has not previously been investigated in human healthy subjects. The interest to investigate these aspects is supported by previous studies which have shown that oxidative modifications of LDL play an important role in the pathogenesis of atherosclerosis (Diaz, Frei, Vita, & Keaney, 1997). In fact oxidixed LDL (ox-LDL) are taken up by scavenger receptors on macrophages and contribute to the formation of foam cells and atherosclerotic lesions (Hulthe & Fagerberg, 2002; Meisinger, Baumert, Khuseyinova, Loewel, & Koenig, 2005; Toshima et al., 2000). In addition, lipid oxidation products are involved in the formation of mutagenic DNA adducts, which may contribute to carcinogenesis (Chung, 1996). Studies in vitro have shown that dietary antioxidants such as ascorbic acid, vitamin E and  $\beta$ -carotene prevent LDL oxidation (Frei, Keaney, Retsky, & Chen, 1996). In vivo studies, however, have yielded contradictory results. Some studies have reported that β-carotene supplementation inhibited LDL oxidation (Levy, Kaplan, BenAmotz, & Aviram, 1996; Nyyssonen, Porkkala, Salonen, Korpela, & Salonen, 1994), whereas others did not find an inhibition of LDL oxidation (Gaziano et al., 1995; Reaven, Khouw, Beltz, Parthasarathy, & Witztum, 1993).

Our aim was to evaluate the bioavailability of carotenoids in black and red cabbage and their effect towards oxidative stress of LDL. Therefore, we enrolled 38 volunteers whose diets were supplemented for 2 weeks with a daily portion of an experimental ready-to-eat product containing 80% of black cabbage (Brassica oleracea L. var. acephala subvar. Laciniata L or Nero di Toscana) and red cabbage (Brassica oleracea L. var. capitata L.f. rubra). Moreover we studied the effect of the intake of the product on the markers of cardiovascular disease blood total cholesterol (TC) and LDL-cholesterol (LDL-C).

#### 2. Methods

#### 2.1. Subjects

The study was conducted during September–November 2012. The inclusion criteria for subjects were: not taking vitamins, minerals, or other types of supplements during the previous Table 1 – Median, first to third quartiles of demographic and anthropometric characteristics, plasma lipid profile (total cholesterol, HDL-C, LDL-C, triacylglycerol) and plasma levels of glucose, carotenoids (lutein and  $\beta$ -carotene), total antioxidant capacity and biochemical markers of oxidative damage (ox-LDL, ox-LDL/LDL-C, lipid hydroperoxides) of subjects at baseline.

Variable	n	Median (1st–3rd quartile)
Age (years)	38	42 (27.25–49.5)
BMI (kg/m²)	36	24.2 (21.18–25.59)
Total cholesterol (mmol/L)	38	5.03 (4.28–5.22)
HDL-C (mmol/L)	38	1.4 (1.22–1.6)
LDL-C (mmol/L)	38	2.92 (2.24–3.32)
Triacyglycerols (mmol/L)	38	0.87 (0.7–1.25)
Glucose (mmol/L)	38	5.16 (4.8–5.44)
Lutein (µg/mL)	23	0.26 (0.2–0.29)
β-carotene (µg/mL)	21	0.36 (0.22-0.44)
Total antioxidant capacity	38	16110 (13860–18410)
(µmolTE/L)		
ox-LDL (U/L)	38	46.12 (28.99–56.61)
ox-LDL/LDL-C (U/mmol)	36	15.96 (12.33–17.65)
Lipid hydroperoxides (µmol/L)	38	2.52 (1.86–3.33)

BMI, body mass index; LDL-C, cholesterol associated with low density lipoproteins; HDL-C cholesterol associated with high density lipoproteins; ox-LDL, oxidized LDL.

2 months; no-smoking; BMI within the normal range according to the World Health Organization criteria (18.5–25 kg/m<sup>2</sup>) and normal biochemical and haematological profile (serum cholesterol <6.8 mmol/L, serum triacylglycerols <2.8 mmol/L, glucose <6.11 mmol/L). The exclusion criteria were: diagnosed diseases such as allergies, cancer, diabetes, obesity, hypertension, mental diseases, gastrointestinal or renal diseases, as well as intake of drugs related to these pathologies, alcohol consumption >30 g/day, vegetarian diet. None of the female subjects was pregnant or lactating. Volunteers were recruited in the Polytechnic University of Marche (UNIVPM), Italy.

Overall, 38 subjects participated in the study, 61% being females. The median age was 42 years. The body mass index (BMI) was within the normal range (Table 1). The intervention phase consisted of a 2-week period which included daily consumption of a portion (300 g) of a frozen product containing black cabbage (Brassica oleracea L. var. acephala subvar. Laciniata L or Nero di Toscana) and red cabbage (Brassica oleracea L. var. capitata L.f. rubra) supplied by Italsur srl (Notaresco, Teramo, Italia).

Intake of the *Brassica* vegetables was included in the normal daily diet and no specific time of consumption or accompanying meal was established. The subjects were recommended to maintain their habitual dietary intake (especially as regard to their consumption of a provided list of foods with high carotenoid, polyphenol and vitamin C contents) and their usual physical activity or other lifestyle habits. Moreover, they were requested to record any sign of illnesses, medications, and any deviations from their experimental diets. Each subject was asked to complete a 15-day dietary record before and during the study intervention to evaluate their energy and nutrient intake. The energy and nutrient contents were estimated by the software "MetaDieta," using Italian food composition tables (MetaDieta software version 3.1, METEDA, Ascoli Piceno, Italy).

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