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Antioxidative properties of chloroplast concentrates obtained by various methods from lucerne juice

B. Baraniak and M. Karaś

Department of Biochemistry and Food Chemistry, Lublin Agricultural University
Akademicka 15, 20-934 Lublin, Poland

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ABSTRACT

The antioxidative properties and chlorophyll contents of protein concentrates obtained by various methods from lucerne were compared. Chloroplast concentrates of lucerne juice (var. *Kleszczewska*) were precipitated by centrifugation (12 000 g), heating (55°C), or with flocculants (Magnafloc LT-26 and Superfloc A-115). The highest antioxidative activity was obtained in the concentrate precipitated by Superfloc A-115 after 24 h incubation with linoleic acid. The highest chlorophyll amount was extracted from the concentrate precipitated by centrifugation; the lowest, from the preparation coagulated with Superfloc A-115.

KEY WORDS: leaf protein concentrate, antioxidative activity, chlorophyll

INTRODUCTION

Production of protein concentrates from green parts of plants was developed to obtain an additional source of protein for animal and human nutrition. Due to the high costs and small yield of the cytoplasmic fraction, such preparations are not produced on an industrial scale. In many countries (USA, France, Hungary, Australia), however, chloroplast concentrates are produced for animal feed supplementation. Their high production costs are justified by their high specific physiological activity. Studies conducted in Japan and Australia (Ostrowski-Meissner, 1993) proved the hypocholesterolemic activity of the commercial preparation, Quintessence®, obtained by a thermal method from lucerne juice. Administration of concentrates in chicken diets lowered plasma cholesterol levels by 27.5% and

triacyloglycerols by 34.3%. Ostrowski-Meissner (1993) proposed a new name, PLEX (pigmented leaf extracts), for the traditional leaf protein concentrate LPC. The name is to underscore the physiological meaning of the compounds co-precipitated with proteins, especially natural plant pigments. Those compounds have the ability to neutralize free radicals and are therefore of great importance in the prevention and treatment of many diseases. In addition to the type of raw material, the method used to concentrate the coagulation is very important in determining the content of particular components in protein preparations (Hernández et al., 1988; Correa et al., 1989; Baraniak, 1992; Ohshima et al., 1996). In the present paper, the influence of the juice protein coagulation method on the antioxidative properties of preparations was studied.

MATERIAL AND METHODS

The material used was fresh lucerne (var. *Kleszczewska*) harvested in the pre-flowering phase. The green forage had previously been crushed in a screw press. The juice was extracted by pressing the green fodder in an expeller and fibrous material remaining in the juice was filtered off through a 0.15 mm = 100 mesh sieve.

Chloroplast particles in the juice were precipitated by the following methods:
- centrifugation (12 000 g)
- heat treatment (55°C)
- flocculation of alkalized juice (pH = 7.5)
- Magnafloc LT-26
- Superfloc A-115

Precipitated chloroplasts were washed twice with distilled water, dried at 50°C, and as chloroplast concentrates stored at a temperature of 2-8°C.

Proteins of chloroplast concentrates were fractionated into those:
- in soluble in phosphate buffer pH 7.0 (-ml/-g) by shaking (2x2 h)
- in soluble in 0.1 M NaOH (-ml/-g) by shaking (2x1 h)

Potential antioxidative substances in chloroplast concentrates, and in fractions of concentrates were extracted by an acetone water solution (4:1 v/v) were studied. The procedure was the following : 30 ml extraction solution was added to 0.4 g samples that were next shaken for 1 h and centrifuged for 10 min (12 000 g); the supernatant was collected and the sediment was again extracted. The process was carried out three times; the combined supernatants (extracts) were adjusted to a final volume of 100 ml.

Antioxidative activity as the ability to form conjugated diene compounds during oxidation was determined spectrophotometrically at 234 nm according to the method of Lingnert et al. (1979). The standard linoleic acid emulsion was pre-

pared by mixing 2 ml 10 mM linoleic acid (Sigma) with Tween 20 (2 ml) and dissolved in phosphate buffer (pH 7.5).

Test samples (0.1 ml of extract) were added to 0.2 ml linoleic acid emulsion and test tubes were incubated in darkness at 37°C for 12, 24 or 36 h. Parallel controls in which extracts were replaced by 80% aqueous acetone were run. After each time period, the reaction mixture was mixed with 2 ml of methanol and 6 ml 60% aqueous methanol and absorption at 234 nm was measured.

The antioxidative activity (AOA) was expressed as the following:

$$AOA=[\Delta A_{234(c)} - \Delta A_{234}]/ \Delta A_{234(c)}$$

where:

ΔA_{234} – increase in absorption during incubation of the experimental sample

$\Delta A_{234(c)}$ – increase in absorption of the control

The chlorophyll content in the obtained acetone extracts was also measured spectrophotometrically (Arnon, 1949; Holden, 1965).

RESULTS AND DISCUSSION

The importance of compounds possessing antioxidative properties in the struggle against cancer is based on prevention of premutagenic DNA changes (Lachance, 1994). Hence, for several years scientists have been focusing on the antioxidative potential of foodstuffs of plant origin. Such properties are possessed by compounds present in seeds (Mehta et al., 1994; Tian and White, 1994; Tsuda et al., 1994), vegetables (Tsushida et al., 1994; Łatosz et al., 1997), and other plant parts (Larson, 1988).

Their biological effectiveness depends on: the type and quality of the raw material and the technical conditions of processing. The correlation between biological activity and its analytical measurements depends on the method applied– mainly on the solvent system used in the extraction (Mehta et al., 1994).

Carotenoids and polyphenols are commonly believed to be the main constituents responsible for antioxidative properties. Comparisons of the concentrations of these compounds in concentrates with values of antioxidative potential do not prove a clear relation, but in some cases a good correlation was obtained (Baraniak, 1997). Concentrates precipitated with Magnafloc LT-26 contained low levels of antioxidants, and in 24 h incubation, which is optimal for this method (Lingnert, 1979), also showed low antioxidative activity (Figures 1 and 2).

Lower values of antioxidative activity were obtained for preparations in which some constituents were washed out by fractionation of concentrates with phosphate buffer or sodium hydroxide and these activities were less dependent on incubation time (Table 1).

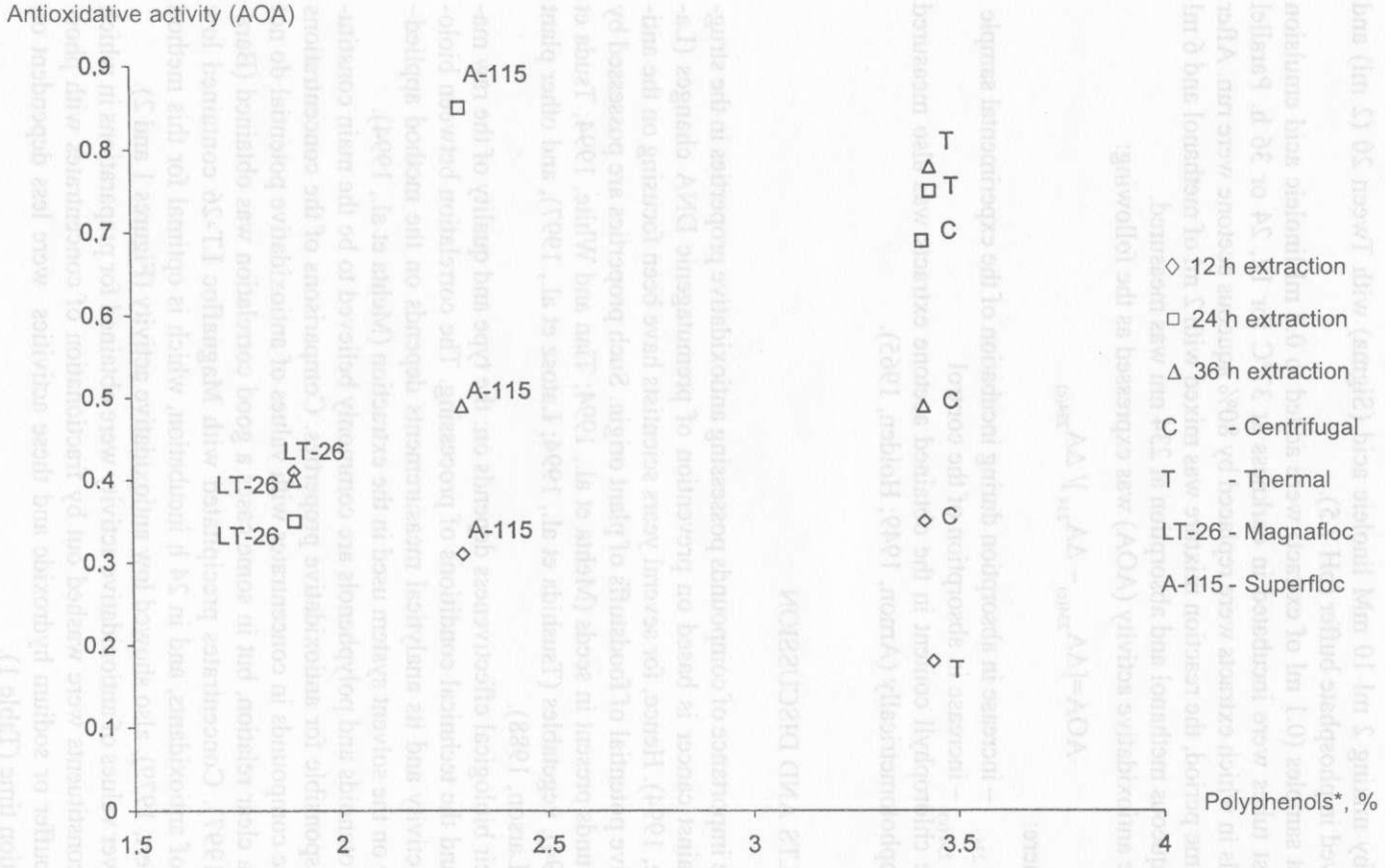

Figure 1. The relationship between antioxidantive activity and polyphenols content in proteins concentrates obtained by varius methods
* Data from Baraniak (1997)

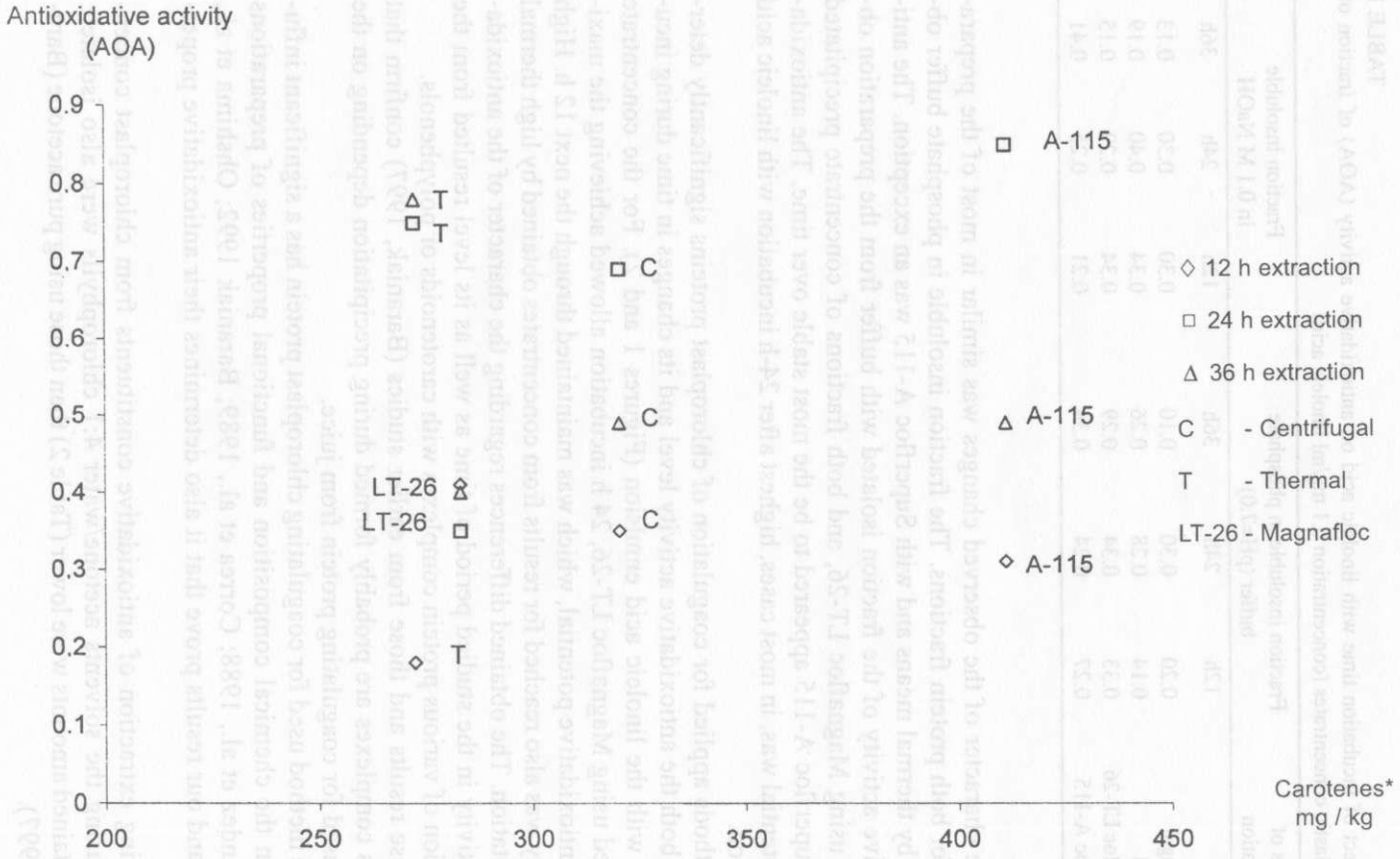

Figure 2. The relationship between antioxidantive activity and carotens content in proteins concentrates obtained by varius methods
* Level of carotens was published earlier (Baraniak, 1997)

TABLE 1

The effect of incubation time with linoleic acid on antioxidative activity (AOA) of fraction of chloroplastic concentrates (concentration 0.1 mg/ml linoleic acid)

Methods of precipitation	Fraction insoluble in phosphate buffer (pH=7.0)			Fraction insoluble in 0.1 M NaOH		
Time	12h	24h	36h	12h	24h	36h
Centrifugal	0.20	0.30	0.10	0.30	0.30	0.13
Thermal	0.14	0.28	0.26	0.34	0.40	0.19
Magnafloc LT-26	0.33	0.34	0.29	0.34	0.29	0.15
Superfloc A-115	0.27	0.24	0.34	0.21	0.25	0.41

The character of the observed changes was similar in most of the preparations for both protein fractions. The fraction insoluble in phosphate buffer obtained by thermal means and with Superfloc A-115 was an exception. The antioxidative activity of the fraction isolated with buffer from the preparation obtained using Magnafloc LT-26, and both fractions of concentrate precipitated with Superfloc A-115 appeared to be the most stable over time. The antioxidative potential was, in most cases, highest after 24-h incubation with linoleic acid emulsion.

Methods applied for coagulation of chloroplast proteins significantly determined both the antioxidative activity level and its changes in time during incubation with the linoleic acid emulsion (Figures 1 and 2). For the concentrate obtained using Magnafloc LT-26, 24 h incubation allowed achieving the maximum antioxidative potential, which was maintained through the next 12 h. High stability was also reached for results from concentrates obtained by high thermal precipitation. The obtained differences regarding the character of the antioxidative activity in the studied period of time as well as its level resulted from the formation of various protein complexes with carotenoids or polyphenols.

These results and those from earlier studies (Baraniak, 1997) confirm that various complexes are probably formed during precipitation depending on the agent used for coagulating protein from juice.

The method used for coagulating chloroplast protein has a significant influence on the chemical composition and functional properties of preparations (Hernández et al., 1988; Correa et al., 1989; Baraniak 1992; Ohshima et al., 1996) and our results prove that it also determines their antioxidative properties.

During extraction of antioxidative constituents from chloroplast concentrates using the solvents acetone/water 4:1 chlorophylls were also isolated. The obtained amounts were lower (Table 2) than those using pure acetone (Baraniak, 1997).

The chlorophyll content was significantly different among the preparations under study. Their highest amount was extracted from concentrates obtained by centrifuging (Table 2), the lowest from preparations flocculated with Superfloc A-115. The percentage of chlorophyll „a" in total chloroplast chlorophyll concentrate was the highest in the thermally precipitated preparation, the lowest, in the centrifuged one (Table 2).

Chlorophylls are stable only in the natural form in which they occur in chloroplasts: bound to proteins, phospholipids or other constituents. Disturbing the native structures during technological processes (heating, grinding, dissolving) causes colour changes due to chlorophyll transformations (Wilska-Jeszka, 1994). The chlorophyll content in protein concentrates can be modified by introducing chosen chemicals into ground raw material (Baraniak et al., 1990) or extracted juice (Baraniak, 1996). Our results confirm earlier observations of Baraniak (1997) that the concentrate coagulation method determines the chlorophyll content as well. In the present paper, no correlation was found between the chlorophyll level in extracts and their antioxidative potential. Our results indicate that the ratio of both chlorophyll forms rather than their content in extracts affects antioxidative activity.

TABLE 2

The chlorophyll content of chloroplastic concentrates dry matter (mg/kg) obtained from lucerne juice by various methods

Methods of precipitation	Fraction of chloroplastic concentrate								
	chloroplastic			insoluble in phosphate buffer (pH=7.0)			insoluble in 0.1 M NaOH		
	chlorophyll			chlorophyll			chlorophyll		
	„a"	„b"	„a" % of total	„a"	„b"	„a" % of total	„a"	„b"	„a" % of total
Centrifugal	1.09	0.98	53	3.35	1.89	64	0.77	0.22	78
Thermal	1.46	0.53	73	3.19	2.76	54	0.76	0.63	55
Magnafloc LT-26	0.90	0.43	68	2.94	1.03	74	0.76	0.15	84
Superfloc A-115	0.48	0.26	65	2.52	1.39	65	0.74	0.60	55

CONCLUSIONS

The concentration of carotenoids and polyphenols affects the level of antioxidative activity, but there is no linear relation when it is measured by oxidation of linoleic acid emulsion. The antioxidative potential of chloroplast concentrates depends on the method applied to obtain them and is more dependent on the ratio of „a" and „b" chlorophylls than on the total amount of these pigments.

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STRESZCZENIE

Właściwości antyutleniajace koncentratów chloroplastycznych otrzymanych z soku lucerny różnymi metodami

Porównano właściwości antyutleniające koncentratów chloroplastycznych z lucerny otrzymanych różnymi metodami. Koncentraty chloroplastyczne z soku lucerny (odm. *Kleszczewska*) otrzymywano poprzez wirowanie (12 000 g), termicznie (55°C) lub wytrącanie flokulantami Magnafloc LT-26 i Superfloc A-115. Największy efekt antyutleniajacy stwierdzono w koncentracie wytrąconym flokulantem Superfloc A-115 po 24-godzinnej inkubacji z kwasem linolowym. Najwiekszą ilość chlorofili wyekstrahowano natomiast z koncentratu otrzymanego poprzez wirowanie, najniższą zaś z preparatu koagulowanego flokulantem Superfloc A-115.