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CHEMICAL CONSTITUENTS AND NUTRITIVE VALUE OF 6 LOCAL VEGETABLE LEAVES BY-PRODUCTS

Rashad, Mona M., S. A. Moharib and Hala M. Abdou
Biochemistry Department, National Research Centre, Dokki, Cairo, Egypt.

ABSTRACT

Leaf protein concentrates (LPC) of cabbage (*Brassica oleracea),*sugar beet *(Beta vulgaris),* alfalfa *(Medicago sativa),* turnip*(Brassica rapa),*spinach*(Spinacia oleracea) and a*rtichoke*(Cynara scolymus)* were extracted and determined. Amino acid composition of the obtained LPC ،s was balanced and exceeded values recommended by FAO to serve as a high-quality food. Essential amino acid levels in the 6 leaf proteins obtained were compared favourably with human (FAO ref. Patterns) and chick requirements, the results indicating that all LPC ،s contained well above the required amounts of preschool child and for chicks,except the amount of methionine,lysine and isoleucine.

The deproteinized juice (whey) which pose serious disposal problems were tested as possible substrates for utilization of different microorganisms. All the tested whey contained reasonable amounts of sugar and nitrogen which could be used as such as a media. Six yeast strains and two edible fungi can grow well on the 6 tested wheys except *Candida maltosa* and *Lentinus edodes* failed to grow on *Cynara scolymus.* and *Brassica oleracea* whey could be considered as the most potential substrate for growing the 8 organisms used in this study,

The LPC produced in the present study have suitable amounts of required essential amino acids good nutritional values, when incorporated into animal foods and human feeds. Also the wheys were used as substrates for growing microorganisms and production of protein and other useful metabolites having nutritional and biological values.

Keywords: Bioconversion - Leaf protein concentrate - Whey - Amino acids-Nutritive value.

INTRODUCTION

Food consumed by a great majority in developing countries oftenly deficient in proteins, both quality and quantity. Many scientists working in the field of food technology had advocated the use of plant proteins in human nutrition. Leaves are potentially the most abundant source of edible protein (Pirie,1987;Rewatre,1989 andRashad,1994). Therefore, leaf protein concentrate (LPC) have been advocated as one of the additional sources for meeting the global shortage of food and feed protein (Liu and Yang,1979; Fantozzi,1985; El-Fouly *et al*. 1987;Youssef *et al.* 1987; El-Baz *et al*.1987,1988; Carleson and Hanczakowski, 1989; Abu-Salem and Ibrahim 1989 and Rashad, 1994). Many investigators reported that the LPC were extracted from different plant leaves (Leucaena, Clover, Lucerne, artichoke, turnip, Maize clover and sugar beet),in a purified form, by grinding and pressing, heating the resultant juice to 80-95˚C to coagulate the protein and separating it by filtration. They reported also that this process extracted about 50 % of the total protein (Pirie ,1987;Youssef *et al*. 1987, Merodio and Sabater 1988; Joshi 1985; Rewatre 1989; Antonov and Tolestoguzov 1990;

Stefanis *et al*. 1990; Chanda *et al*. 1990a; Farinu *et al*.1992 and Rashad, 1994). The nutritional properties of protein fractions as well as the amino acids of different leaves species have been discussed extensively (Youssef *et al*. 1987; Kobayashi and Itoh 1989; Abu-Salem and Ibrahim 1989; Chanda *et al*. 1990a; Stefanis *et al*. 1990; Cid *et al*.1991; Baraniak and Bubic 1991; Farinu *et al*. 1992; Pasieka et al. 1992; Dewanji,1993 and Rashad 1994)

During the production of vegetable protein from leaf Juice, then fibre and a liquid waste, deproteinized leaf Juice (DLJ), is generated. This by-product, composed of sugars, amino acids, lipids, minerals and vitamins, present a serious treatment problem because of its high chemical and biochemical oxygen demands and low pH. Microbiological transformation of these nutrients into useful biomass would be beneficial to avoid local pollution as well as for economic reasons (Chanda 1982; Chanda et al. 1980,1984,1990 a, b; Pirie,1987;; Hang and Woodams1990; Pandey *et al*. 1991 and Overchenko *et al*.1998).

Therefore, the aim of the present investigation was to study the extractability of protein from different Egyptian leaves as well as the chemical composition of the leftover by-products formed was also analyzed in an attempt to study its score for utilization by several microorganism.

MATERIALS AND METHODS

Materials

The leafy by-products materials of cabbage (*Brassica oleracea), s*ugar beet *(Beta vulgaris),* alfalfa *(Triflolium alexandrinum),* turnip*(Brassica rapa),*spinach*(Spinacia oleracea)* and artichoke *(Cynara scolymus)* were collected from the local market.

Organisms

Yeast strains (*Hansenula polymorpha*, *Candida guilliermondii* (Y-2075), *Candida boidinii*(Y-4235), *Pichia Pinus*, *Trichodermia reesi* and *Candida maltosa* (R42) were obtained through the courtesy of Prof.Sidney Crow, Biol. Department, Georgia state University, Georgia, USA. Strains were routinely stored in YM stock medium.

Mushroom species (*Pleurotus ostreatus* NRRL-O366, *Lentinus edodes* NRRL-22663) obtained from Agricultural Research Service (Peoria, II) were maintained in large tubes containing agar-potato dextrose (Jodon and Royse,1979).

Methods

Leafy by-products protein were obtained according to the procedure described by Goel *et al*.(1978),by crushing known weight of leaves with known volume of water in domestic mincer, squerzing the slurry through cloth,heating the juice extract at 80˚C to coagulate the protein, which has laterly filtered, washed with hot distilled water, dried in freeze dryer and finally powdered to yield leafy protein concentrate (LPC),stored till used.

Analytical Procedures

Moisture and dry weight were determined gravimetrically (A.O.A.C.,1980). Crude protein in LPC was determined by the Kijeldahl method as N x6.25 (Loiseleur, 1963). Total carbohydrates were hydrolysed by2M H_2SO_4 and then estimated in the hydrolyzate according to the method devised by Dubois *et al*.(1956). The amino acid composition of LPC hydrolyzates (acid hydrolysed with 6N HCL at 110˚C for 22 h (Block and Bolling, 1951) was determined with an HPLC amino acid analyzer Eppendorf LC 3000. Statistical analysis was done according to Fisher (1970).

Cultivation and fermentation

Fermentation of the whey (deproteinized juice) of each plant used in this study was done in a test tube containing 5 ml of the medium and autoclaving for 20 min. at 121˚C. The inoculum of 6 yeast strains separately were made in a sterilized saline solution for 24-40h. The screening test was done by transferring 0.1ml of the inoculum (starting absorbance A ranged from 0.05-0.15 at 610 nm) to 5 ml of sterilized whey at 29±1˚C on a rotary shaker (150 r.p.m.) for 4 days. The cell growth was estimated by measuring the absorbance of the culture broth at 610 nm. The inoculum of the 2 edible fungi consisted of 1 cm^2 disc of mycelium and agar obtained by using a sterile cork borer,was transferred to the sterilized 10 ml of the whey medium in the large test tubes. Static cultures at 25 ˚C for 7 days were achieved. The mycelia was collected by filtration and washed with distilled water then dried in freeze dried and dry weight was calculated gravimetrically.

RESULTS AND DISCUSSION

The major chemical constituents of the tested samples were given in table (1). The water absorption capacity is slightly differed in all the tested samples (83.70%-87.37%) except that of alfalfa (71.00%). These results are in accordance with those obtained by El-Fouly *et al*. (1987) and Rashad (1994) they used Turnip,Sugar beet crops. Extraction of proteins from the leaves of the 6 vegetable crops was done and the results in table (1) revealed the presence of different amount of protein ranged from 36.70 g/kg-90.00 g/kg except that obtained from *Spinach* which was 10.17 g/kg.

Table (1): Chemical constituents of by-products of leaves of 6 vegetable crops.

Vegetable crops	Leaf moisture %	g/kg fresh wet leaves		Liquid waste (whey)	
		Protein (LPC)	Residual fibre	Total sugar g/l*	Total nitrogen g/l
Cabbage	87.37±1.01	90.00±0.86	630.00±6.54	36.4±1.04	1.80±0.07
Sugar beet	83.74±1.10	36.70±1.06	410.00±5.35	12.30±1.04	1.25±0.08
Alfalfa	71.00±1.14	59.85±0.92	710.00±8.58	27.00±1.02	1.54±0.04
Turnip	85.99±1.52	55.97±0.86	512.00±4.63	17.5±0.08	1.68±0.05
Spinach	83.79±0.09	10.17±1.82	150.00±2.86	15.50±1.06	0.86±0.02
Artichoke	85.00±1.22	55.00±0.64	571.00±5.60	12.2±0.05	1.60±0.04

*** Glucose equivalent.**

Mean values of 5 samples (mean ± S.E.).

The same variations were obtained with respect to the residual fibre and also *Spinach* was the lowest one containing fibre (150.00 g/kg). These results are with in the range those obtained by several workers (El-Fouly *et al*. 1987; Abu-Salem and Ibrahim, 1989; Rashad,1994; Jwanny *et al*. 1996; El-Beih *et al*. 1996 and Moharib,1997).

Amino acid composition of leaf protein concentrates

Amino acid composition is one of the most commonly used criteria to judge the nutritional value of a protein. Table (2) shows the amino acid profile for the LPC's of the tested samples. Data in table (2) revealed that there is uniformity the total amino acids of the 4 crops (Cabbage, *Alfalfa, Turnip and Artichoke)* which ranged from 66.74 g% to 76.66 g%. This general similarity was confirmed by other workers (Byers,1971 and 1975; El-Hennawy *et al*. 1977; Pirie,1987; El-Fouly *et al*. 1987; Dewanji,1993 and Rashad,1994). At the same time, some differences occurred in the content of S-containing amino acids and aromatic amino acids when compared with egg FAO standard (Delaney *et al*. 1975). The other 2 crops (*Sugar beet* and *Spinach),* showed the higher amino acids content (86.94 % and 92.97 % respectively). Their LPC's showed nearly the same values of total amino acids with slight variations of the corresponding individual amino acids except methionine and histidine in *Sugar beet* crop which was lower than the corresponding value in *Spinach* by 50 % and 25 % respectively (Table 2). In spite of *Spinach* leaves were the lowest crop of the tested one in protein content (10.17 g/kg leaves) as shown in Table (1), its LPC's is the best one as their amino acid content which recorded higher value (92.97 %) containing high total essential amino acids (about 39.00 %). Their content of essential amino acids was in the same level with those obtained with other workers with various crops (Joshi,1985; Chabaev *et al*.1990; Baraniak and Bubic,1991; Farinu *et al*. 1992 and Rashad,1994). Several workers suggested supplementing LPC with methionine to improve its nutritive value, or to combine it with wheat flour, or diets (Byers,1971; Joshi,1985; El-Fouly *et al*. 1987; Jham *et al*. 1989; Stefanis *et al.* 1990 and Olvera *et al.* 1990). A comparison with standard was done to provide a means of predicting the contribution of these vegetable leaf proteins toward meeting human/animal amino acid requirements. The essential for a particular species are not exactly the same for other species. The requirement varies depending on the age and species as is evident from Table (2). The requirement of essential amino acids is critical in the nutrition of nonruminants such as humans and chicks as they do have the ability to synthesize certain essential amino acids (Banerjee,1988). On comparing the essential amino acids of the 6 crops used in this study with those required for humans (FAO/WHO,1985) and for chicks (NRC,1984), the data in Table (2) revealed that all LPC's contained well above the suggested pattern for requirement for adults and satisfied the required amounts for preschool children and chicks except the amount of methionine, isoleucine and lysine were lower than that required for chicks, therby indicating that they could be used as a food / feed sources.

The chemical constituents of the liquid waste left after protein extraction revealed that in general, the collected whey of all the 6 crops

contained reasonable amounts of total sugar and nitrogen (Table 1). *Cabbage* was the best one for as it contained large amount of sugar (36.00 g/l juice), followed by *Alfalfa* (27.00 g/l juice). Also, C/N ratio of *Cabbage, Alfalfa and Spinach* were equal to about 20:1,while the other 3 crops contain nearly the same ratio, 10:1 (Table 1). Similar results was obtained for *Sugar beet* with the author Chanda *et al.* (1990b), while with respect to *Turnip*, the

Table (2): Amino acids profile in hydrolysates of LPC of the 6 vegetable crops.

Amino acids	g amino acids / 16 g N [a]						Whole egg FAO Standard [b]	FAO/WHO reference pattern [c]		NRC [d] requirement for chicks
	Cabbage	Sugar Beet	Alfalfa	Turnip	Spinach	Artichoke		Preschool child	Adult	
Aspartic acid	8.99	10.60	8.22	7.43	10.80	8.11				
Serine	4.02	5.60	3.36	4.01	4.88	4.17				
Glutamic acid	10.03	11.91	7.96	9.46	12.04	8.98				
Proline	3.91	5.80	4.17	5.01	5.93	3.01				
Glycine	5.19	7.75	4.58	5.47	6.31	4.95				
Alanine	6.41	5.75	4.57	5.35	6.50	5.30				
Cystine	0.13	0.53	0.00	0.30	0.47	0.43	2.40			
Threonine	3.81	4.50	3.43	4.04	4.93	3.63	5.10			
Valine	3.61	3.17	3.10	3.41	4.07	3.46	7.30	3.40	0.90	3.50
Methionine	1.36	0.72	1.07	0.74	1.79	0.71	3.20	3.50	1.30	4.30
Isoleucine	2.77	2.42	2.25	2.15	2.97	2.63	6.60	2.50[e]	1.70[e]	3.80[e]
Leucine	6.16	5.11	5.63	3.30	6.80	5.95	8.80	2.80	1.30	3.80
Phenylalanine	3.04	4.90			5.28	3.58	5.80	6.60	1.90	7.00
Lysine	4.73	3.77	3.87	4.20	4.32	5.18	6.40	6.30f	1.90f	6.50f
Tyrosine	3.10	4.53		4.95	4.60	3.02	4.20	5.80	1.60	5.50
Histidine	3.74	2.96	3.96	3.39	4.23	3.67				
Arginine	4.71	5.96	2.82	3.81	6.19	3.83				
NH4	0.95	0.98	3.07	4.68	0.86	0.93				
Total amino	76.66	86.94	3.96	0.75	92.97	71.54				
acids	32.32	32.08	0.72	72.45	38.99	31.83				
Total essential			66.74	29.99						
Amino acids			29.20							

a: 16 g N equivalent 100 g crude protein. b:Delaney *et al*. (1975). C:FAO/WHO (1985).
d : NRC (1984). e: Requirements for methionine plus cystine.
f : Requirements for phenylalanine plus tyrosine.

present result was nearly double than that obtained by Chanda *et al.* (1990b*).* Several workers reported that the C/N ratio (20:1 or 10:1) was suitable for growing different microorganisms (yeast and fungi) to yield useful metabolites Rashad *et al.*(1990); Chanda *et al*. (1990b) and Jwanny *et al*. 1989 and 1995).

Chanda *et al*. 1990a and Sim and Hanger,1995 fortified beet whey, turnip whey and saurkraut brine (a by-product of cabbage processing industry) used with sucrose or molasses to give 10-15 % total sugar in the cultivation medium to produce protein,citric acid or other useful metabolites. Also Chanda, (1992), use the deproteinized juice from two aquatic plants as a medium for production yeast protein.

So, as these liquid wastes (whey) collected in the present studies contains reasonable level of C and N, preliminary experiments will be done to use each of these waste (as it is) as a media for growing 8 different organisms for 4 days for yeast and 7 days for edible fungi. Data represented

in Table (3) showed the ability of the tested organisms to grow on the 6 different leaf by-products whey which varied with different degree. All the tested organisms could grow on the 6 by-products whey except *C.maltosa* and *L.edodes* failed to grow on *Artichoke* whey.

Table (3): Ability of 8 microbial strains to grow on 6 different leaf by-products plant whey.

Microorganisms*	*Cabbage*	*Sugar beet*	*Alfalfa*	*Turnip*	*Spinach*	*Artichoke*
Hansenula polymorpha Candida	+++	+	+++	++	+	+
guilliermondii	+	+++	+++	++	++	+++
Candida boidinii	+++	++	+++	++	+++	+++
Pichia Pinus	+++	++	+++	++	++	++
Trichodermii reesi	+++	+++	++	++	+++	+++
Candida maltosa	+++	++	+++	++	+	--
Pleurotus ostretus **	+++	+++	++	+++	++	+
Lentinus edodes **	++	++	+	+	+++	--

+ :Scantly growth. **++ :Moderate growth.**
+++:Abundant growth. *** :Growth for 4 days.**
**** :The 2 strains grown for 7 days.**

The strains *C. boidinii* (Y-4235) and *Trichodermii reesi* have higher ability to grow on the 6 collected wheys, especially on *Cabbage, Spinach* and *Artichoke* wheys. Analysis of the available data given in Table (3) indicated that *Cabbage* whey was considered as a potential substrate for growing microorganism followed by alfalfa whey. Further studies will be done for bioconversion of *Cabbage* whey to useful metabolites.

From the previous discussion, it is suggested that, leaf protein obtained from the leafy-by-products of the 6 tested crops, could be recommended as a potential source of a higher quality protein. Simultaneously to reducing BOD levels which could cause pollution problems and for economic reasons ,as it would be advantageous to use the fibre in feeding and of the utilizing wheys as a media for producing some useful metabolites.

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المكونـات الكيميائبيـة والقيمـه الغذائيـه لمخلفـات 6 أنـواع مـن أوراق الخضـروات المحليـة

مـني محمـد رشـاد وسـوريال عـدلي محـارب وهالـه محسن عبـده
قسم الكيمياء الحيويه - المركز القومي للبحوث - الدقي – القاهره – مصر

تـم اسـتخلاص وتركيـز البـروتين مـن أوراق كـل مـن الكرنـب وبنجـر السـكر والبرسـيم واللفـت والسبانخ والخرشوف. وتبين أن البروتينات الناتجه تحتوى على نسبة من الاحماض الامينيه تتفق مع الجداول الخاصـة بمنظمة الفاو حيث انها تعتبرها نوعية عاليه من الغذاء.كما تم تقدير الاحماض الامينيه الضروريه فى 6 عينات من البروتينات المستخاصه من أوراق الخضـروات تحت الدراسه وتبين أنها تزيد عن متطلبات منظمـة الفـاو فـى تغذيـة الانسـان والـدجاج تحتـوي علـى الكميـات اللازمـه مـن الاحمـاض الامينيـه الضـروريه واللازمه لغذاء الاطفال والكتاكيت باسـتثناء كمية الميثيونين والليسين والايزليوسين0

وبالنسبه للعصير المتبقى بعد استخلاص وتركيز البروتين (الشرش) والذى يشكل مشاكل بيئيه فى التلوث فقد تم استخدامه كأوساط غذائيه لتنمية الكائنات الدقيقه حيث وجـد أن شرش جميع الاوراق تحت الدراسه تحتوى على كميات مناسبه من السكريات والنيتروجين وقد تم استعماله كما هو كوسـط غـذائى لتنمية 6 سلالات من الخميره وسلالتين من الفطريات ووجد أن جميعـها تنمو علـى تلك الاوساط ماعدا الكانديدا مالتوزا واللانتيناس ايدودس حيث اخفقا فى النمو علي شرش الخرشوف0

لذلك فان البروتين المركز الناتج من هذه الدراسه يحتوى على الكميات اللازمـة من الاحماض الامينيه الضروريه وله قيمه غذائيه عاليه عند تغذيته او اضافته لوجبات غذائية للانسان أوالدواجن وأيضـا فـان الشرش يستخدم كأوساط غذائيه لتنمية الكائنات الدقيقه لانتاج بروتينات ومواد أخرى مفيـدة لها قيمه غذائيه وبيولوجيه .

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