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

Leaf protein concentrates (LPC) of cabbage (Brassica oleracea), sugar beet (Beta vulgaris), alfalfa (Medicago sativa), turnip (Brassica rapa), spinach (Spinacia oleracea) and artichoke (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 poses 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 and Rashad, 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 grading 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;
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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; Sid et al. 1991; Baraniak and Bubic 1991; Farinu et al. 1992; Pasieka et al. 1992; Dewanj, 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, 1990 a, b; Pirie, 1987; Hang and Woodams 1990; 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), sugar beet (Beta vulgaris), alfalfa (Trifolium 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, squering the slurry through cloth, heating the juice extract at 80°C to coagulate the protein, which has latterly 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 Kjeldahl method as N x 6.25 (Loiseleur, 1963). Total carbohydrates were hydrolysed by 2M H2SO4 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 cm² 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.

<table>
<thead>
<tr>
<th>Vegetable crops</th>
<th>Leaf moisture %</th>
<th>g/kg fresh wet leaves</th>
<th>Liquid waste (whey)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein (LPC)</td>
<td>Residual fibre</td>
<td>Total sugar g/l*</td>
</tr>
<tr>
<td>Cabbage</td>
<td>87.37±1.01</td>
<td>90.00±0.86</td>
<td>630.00±6.54</td>
</tr>
<tr>
<td>Sugar beet</td>
<td>83.74±1.10</td>
<td>36.70±1.06</td>
<td>410.00±5.35</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>71.00±1.14</td>
<td>59.85±0.92</td>
<td>710.00±8.58</td>
</tr>
<tr>
<td>Turnip</td>
<td>85.99±1.52</td>
<td>55.97±0.85</td>
<td>512.00±4.63</td>
</tr>
<tr>
<td>Spinach</td>
<td>83.79±0.09</td>
<td>10.17±1.82</td>
<td>571.00±5.60</td>
</tr>
<tr>
<td>Artichoke</td>
<td>85.00±1.22</td>
<td>55.00±0.64</td>
<td>150.00±2.86</td>
</tr>
</tbody>
</table>

* 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 uniformly 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, thereby 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 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

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

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>g amino acids / 16 g N a</th>
<th>Whole egg FAO Standard b</th>
<th>FAO/WHO reference pattern c</th>
<th>NRC d requirement for chicks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cabbage</td>
<td>Sugar Beet</td>
<td>Alfalfa</td>
<td>Turnip</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>8.99</td>
<td>10.60</td>
<td>8.22</td>
<td>7.43</td>
</tr>
<tr>
<td>Serine</td>
<td>4.02</td>
<td>5.60</td>
<td>3.36</td>
<td>4.01</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>10.03</td>
<td>11.91</td>
<td>7.96</td>
<td>9.46</td>
</tr>
<tr>
<td>Proline</td>
<td>3.91</td>
<td>5.80</td>
<td>4.17</td>
<td>5.01</td>
</tr>
<tr>
<td>Glycine</td>
<td>5.19</td>
<td>7.75</td>
<td>4.58</td>
<td>5.47</td>
</tr>
<tr>
<td>Alanine</td>
<td>6.41</td>
<td>5.75</td>
<td>4.57</td>
<td>5.35</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.13</td>
<td>0.53</td>
<td>0.00</td>
<td>0.30</td>
</tr>
<tr>
<td>Threonine</td>
<td>3.81</td>
<td>4.50</td>
<td>3.43</td>
<td>4.04</td>
</tr>
<tr>
<td>Valine</td>
<td>3.61</td>
<td>3.17</td>
<td>3.10</td>
<td>3.41</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.36</td>
<td>0.72</td>
<td>1.07</td>
<td>0.74</td>
</tr>
<tr>
<td>Isolucine</td>
<td>2.77</td>
<td>2.42</td>
<td>2.25</td>
<td>2.15</td>
</tr>
<tr>
<td>Leucine</td>
<td>6.16</td>
<td>5.11</td>
<td>5.63</td>
<td>3.30</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.04</td>
<td>4.90</td>
<td>5.28</td>
<td>3.58</td>
</tr>
<tr>
<td>Lysine</td>
<td>4.73</td>
<td>3.77</td>
<td>3.87</td>
<td>4.20</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>3.10</td>
<td>4.53</td>
<td>4.95</td>
<td>4.60</td>
</tr>
<tr>
<td>Histidine</td>
<td>3.74</td>
<td>2.96</td>
<td>2.96</td>
<td>3.39</td>
</tr>
<tr>
<td>Arginine</td>
<td>4.71</td>
<td>5.96</td>
<td>2.82</td>
<td>3.81</td>
</tr>
<tr>
<td>NH₄</td>
<td>0.95</td>
<td>0.98</td>
<td>3.07</td>
<td>4.68</td>
</tr>
<tr>
<td>Total amino</td>
<td>76.66</td>
<td>86.94</td>
<td>78.96</td>
<td>92.97</td>
</tr>
<tr>
<td>Acids</td>
<td>32.32</td>
<td>32.08</td>
<td>32.72</td>
<td>32.45</td>
</tr>
<tr>
<td>Total essential</td>
<td>99.00</td>
<td>119.04</td>
<td>111.68</td>
<td>125.42</td>
</tr>
<tr>
<td>Amino acids</td>
<td>88.74</td>
<td>86.74</td>
<td>85.96</td>
<td>92.97</td>
</tr>
</tbody>
</table>

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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.

<table>
<thead>
<tr>
<th>Microorganisms*</th>
<th>Cabbage</th>
<th>Sugar</th>
<th>Alfalfa</th>
<th>Turnip</th>
<th>Spinach</th>
<th>Artichoke</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hansenula polymorpha Candida guilliermondii</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Candida boidinii</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Pichia Pinus</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Trichodermii reesi</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Candida maltosa</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>--</td>
<td>++</td>
</tr>
<tr>
<td>Pleurotus ostreatus **</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Lentinus edodes **</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>--</td>
<td></td>
</tr>
</tbody>
</table>


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.

REFERENCES


7234


7237
المكونات الكيميائية والقيم الغذائية لمختلفات 6 أنواع من أوراق الخضروات المحلية

منى محمد رشاد وسوريال عدلي محارب وهاله محسن عبيد
قسم الكيمياء الحيوية - المركز القومي للبحوث - الدقي - القاهرة - مصر

تم استخلاص وتركيز البروتين من أوراق كل من الكرنب ونبجس السكر والبرسيم واللفت والسبانخ والخضروات. وتبين أن البروتينات الناتجة تحتوي على نسبة من الاملاح الأمينية تتفاوت مع الجداول الخاصة بمنطقة الفار حيث أنها تعتبرها نوعية عالية من الغذاء، كما تم تقييم الاملاح الأمينية الضرورية في 6 عينات من البروتينات المستخدمة من أوراق الخضروات تحت الدراسة وتبين أنها تزيد عن متطلبات منظمة الفاو في تغذية الإنسان والنرجاح تحتوي على الاملاح الأمينية الضرورية من الاملاح الأمينية الضرورية واللازم لغذاء الأطفال والكبار، باستثناء كمية الستيروفين والليسين والأمينوسيد.

ويستجيب للعصر المعاصر بعد استخلاص وتركيز البروتين (الشروح) والذي يشكل مشكلة بسيطة في الثلوج فقد تم استخدام كأداة غذائية لขนม البيانية حيث وجد أن شرش جميع الأوراق تحت الدراسة تحتوي على كميات مناسبة من الكربوهيدرات والنهارودين وفقاً لاستعمال كم هو كم سلسة غذائية لفنية 6 سلالات من الخمير وسلالات من النباتات ووجود أن جميعها تنمو على تلك الأوساط ماعدا الكاهننا مالتوز ونبر الرياضيون حيث انها في النمو على شرش الخضروات.

لذلك فإن البروتينات المركز الناجح من هذه الدراسا تحتوي على الاملاح الأمينية الضرورية لفائدة غذائية عالية عند تغذية أو اضافة لوجبات غذائية للإنسان أو الفواكه وأيضا فإن شرش يستخدم كأداة غذائية لفنية الكائنات الغذائية لانه تشتتبري ومواد أخرى مفيدة لها قيمة غذائية وبيولوجية.