



A comprehensive study on the effect of additives on the yield of LPC prepared from *Medicago sativa* Linn

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Abstract

During green crop fractionation, the leaf juice is released after pressing of macerated green foliage. The leaf juice expressed during this process is employed for the preparation of leaf protein concentrate (LPC), a protein-vitamin-mineral rich concentrate suitable for human nutrition. Singh (1962) recorded loss of proteins from the juice extracted from green foliage if it is not immediately processed for the preparation of LPC. It was pointed out that there is breakdown of proteins, appearance of ammonia, fermentative loss of sugars, drop in pH and decrease in the dry matter content when the juice is stored at room temperature for 48 hours. Proteolytic activity and microbial growth in juice deteriorate it. These catabolic reactions during storage of the juice results in low recovery of protein in the LPC with low yield of LPC per unit volume of the juice. It is therefore, suggested that the leaf juice should be employed for the preparation of LPC immediately after its extraction to achieve maximum yield of leaf protein. During present course of investigation effect of additives on the yield of LPC from lucerne juice stored for 48 hours was studied.

Keywords: Lucerne, leaf protein, LPC etc.

Introduction

Storage of juice at 22 °C for over 24 hours results in up to 20% decrease in protein which is recoverable in LPC. Singh (1962)^[9] observed that from 7 to 20% of protein was autolysed when leaf extracts were incubated for 2 hours at 37 °C, and in an extract from young wheat leaves upto 40% of protein was lost in 2 hours. De Fremery *et al.*, (1973)^[2] and Balasundaram *et al.*, (1975) have reported 6 to 8% loss of protein in lucerne juice stored at the temperatures between 17 and 30 °C for 2 hours. De Fremery *et al.*, (1973)^[2] also reported upto 50% reduction in protein from lucerne juice stored at 50°C. Effect of additives on chlorophyll content in wet LPC prepared from juice of *Medicago sativa* Linn., was studied by Sayyed (2010). LPC: A Good Source of Cyanocobalamine (B12), Ascorbic Acid (Vitamin C) and Folic Acid (Vitamin B9) (Ilyas & Badar, 2010a)^[4]. Estimation of Thiamine, Riboflavin and Pyridoxine from LPC of Some Plants (Ilyas and Badar, 2010b)^[5]. Study of LPC and PCR Prepared from Radish (*Raphanus Sativus* Linn.) was carried out by Sayyed (2011)^[7]. Sayyed and Mungikar (2003)^[11] observed the changes in chlorophyll content of lucerne leaf juice during storage. The Use of Deproteinised Leaf Juice (DPJ) in Microbial Biotechnology was studied by Sayyed

and Mungikar (2005)^[12]. Josephin and Sayyed (2005)^[6] has proved that Deproteinised Leaf juice as a medium for fungal growth and for production of Protease.

Sayyed (2013)^[8] observed that production of amylase of DPJ of four different plants. In view of some bioinformatical approach of this work, Ansari and Ilyas (2011)^[1] has performed a comparative study of Protein Structure Visualization Tools for various display capabilities. A comparative study of different properties provided by Protein Structure Visualization Tools (Ilyas and Ansari 2013)^[3]. The reduction in recoverable protein from stored juice has been ascribed to the activity of endogenous proteolytic enzymes, which in juice cause degradation of precipitable protein particles into soluble peptides and amino acids, which in turn cannot be recovered in the precipitation/coagulation procedure (Singh, 1962; de Fremery *et al.*, 1973)^[9, 2]. Work in many laboratories demonstrated enzymatically induced instability of proteins. During present investigation attempts were made to preserve the juice extracted from green foliage of lucerne up to 48 hours. The juice was either stored without any chemical treatment or it

was pretreated with either formic acid, citric acid, ascorbic acid or sodium metabisulphite.

The changes in pH, yield of LPC and chlorophyll content were noted during the preservation of juice.

Materials and Methods

Fresh green foliage of lucerne was harvested early in the morning at a preflowering stage. It was pulped and subsequently pressed and the juice released due to the pressing was collected. The first experiment was undertaken during January 2000 wherein the juice was distributed in 10 conical flasks. Each flask, containing 100 ml juice was plugged with cotton and were left at room temperature for storage. In order to evaluate the effect of additives, the juice was pretreated with 2% NaCl, and in addition with either 2% formic acid, citric acid, ascorbic acid or sodium metabisulphite, before keeping it for storage. The samples of the juice were collected after every 6 hours upto 48 hours. At each collection, pH of the juice was measured using pH meters (Elico Model LI-10T). The sample of 100 ml juice was employed for the preparation of LPC by heat coagulation method (Pirie, 1971) [10]. The LPC was filtered through Whatman filter paper, washed with hot water for minimum 3 times, dried in oven at 65 ± 5 °C till constant weight and the weight of LPC was recorded as the yield of LPC/100 ml of juice.

Results and Discussion

When any biological material is stored *in vitro*, catabolic changes are expected due to microbial and enzymatic activities leading to either fermentation or deterioration. This leads to the change in chemical composition of the material which is desirable in few cases while undesirable in majority of the events. The experiments undertaken during present investigation gives an account of the effect of formic, citric and ascorbic acids as well as sodium metabisulphite along with salt on the chemical changes associated with storage of lucerne leaf juice.

Information on the changes in pH and the yield of LPC during storage of leaf juice for different time intervals is given in Table 1. In untreated (control) leaf juice the initial pH was 5.7 which gradually decreased to 4.8 at the end of 48 hours. Addition of formic acid at the rate of 2% decreased the pH of the juice to 4.3 which further reduced to 4.0 due to the storage for 48 hours. Citric acid was added to the juice at 2% concentration which

resulted into the juice sample with pH value of 5.1; the pH decreased further to 4.4 due to the storage. Ascorbic acid pretreatment to the juice was responsible for reducing the pH to 4.4 and subsequently to 4.0 due to the storage. When sodium metabisulphite was added as an additive the initial pH of the juice slightly decline to 5.3 with further decrease to 4.6 due to its preservation at room temperature. The variation in the pH of the juice recorded after every 6 hours was expressed as coefficient of variation (C.V.), which was maximum (6.1%) in untreated juice, medium (5.2 and 4.5%) in the juice samples treated with citric acid and sodium metabisulphite respectively and minimum (2.7 and 2.9%) in formic and ascorbic acid treated leaf juice samples. Thus formic acid and ascorbic acid successfully prevented fluctuation in the pH during storage with an average value of 4.0%. Thus it can be concluded that pretreatment of juice with either formic acid or ascorbic acid along with NaCl is beneficial in maintaining the juice in acidic condition which is essential for preventing deterioration.

The yield of LPC in case of fresh untreated juice was 1.902 g/100 ml at the end of 48 hours. Treatment of the juice with additives was found to be useful in preventing decrease in the yield of LPC and on an average the yield of LPC/100ml of juice was 3.750 ± 0.157 to 4.043 ± 0.238 g/100 ml. Large variation in the yield of LPC (C.V. = 23.217%) was recorded with untreated leaf juice while the values for C.V. were between 4.1 and 5.8 in case of the juice samples treated with additives.

The overall results presented in Table 1 indicated that pretreatment of leaf juice with additives used during this investigations were effective in preventing the decrease in the recovery of LPC from the juice. It is felt that if the preparation of LPC is delayed by some or the other reasons the use of additives will be useful for maximum recovery of leaf protein from the juice.

Conclusion

During green crop fractionation (GCF) the leaf juice released after maceration and pressing of green foliage is normally employed to prepare leaf protein concentrate (LPC). Preparation of LPC from juice, immediately after its extraction, has been advocated for maximum recovery of high quality of LPC, as the juice is liable for chemical, enzymatic and microbial reactions if its further processing is delayed. Numerous reports indicated catabolic activity in the juice in relation to low recovery of leaf protein, which is of poor quality. Almost all reports suggested

that the LPC should be prepared without delay. However, if it is not possible to prepare LPC immediately after extraction of juice, it is imperative to prevent catabolism, particularly the breakdown of proteins in juice for maximum recovery of LPC. During present study it was observed that additives like formic acid, citric acid, ascorbic acid and sodium metabisulphite, along with salt may also be used to prevent deterioration of juice to some extent.

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