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Effect of Storage Temperature and Light on the Freeze-Dried Amino Acids from Sugar Beet and Sugar Cane Molasses

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ABSTRACT: Molasses is a significant by-product of sugar beet or sugar cane refining industry. In this work, the effects of storage temperature and packaging on the contents of free amino acids (AAs) in sugar beet and sugar cane molasses after freeze drying were inquired. The effect of different variables such as, temperature (4 and 25˚C) and light (Metallized polypropylene container and Normal polypropylene container) were evaluated to determine the optimum condition for storage of AAs molasses. The results showed that metallized polypropylene container was the suitable light container to maintain AAs from sugar beet and sugar cane molasses since AAs might be denatured by the light. Furthermore, low temperature had better influences on AAs during storage since high temperature might destroy AAs therefore AAs might be stabilized for a long time at low temperature. The finding in this study might be employed for other industries such as medical and pharmaceutics industries to store the valuable AAs.

Keywords: *Amino Acids, Freeze Drier, Shelf Life, Sugar Beet Molasses, Sugar Cane Molasses*.

Introduction

Sugar cane (*Saccharum officinarum* L.) and sugar beet (*Beta vulgaris* L. ssp. saccharata) are the most substantial crops for production of sugar. Molasses is the thick, dark, residual syrup from the processing of sugar beet and sugar cane that contains high amount of solids (approximately 80%; Honma, 2012; Samatav and Samatav, 2014; Saric *et al.*, 2016). It mainly consists of around 50% fermentable carbohydrates (sucrose, glucose, fructose) and several nonsugar organic compounds (betaine and other amino acids; minerals and trace elements; vitamins, particularly of the B-group, etc.; Saric *et al.*, 2016; Bernal *et al.*, 2016; Valli *et al.*, 2012).

Amino acids (AAs) have a valuable role in all the living organisms. They are considered the basis of life, functional properties as building blocks of proteins and intermediates in the metabolism. AAs are widely employed in food, medical, cosmetic and pharmaceutical industries due to their

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important edible and commercial values (Bernal *et al.*, 2012; Bernal *et al.*, 2008; Wang *et al.*, 2017; Martin-Orue *et al.*, 1998, Zhu *et al.*, 2015).

Drying as a preservation method is quite important in food manufacturing (Hassan *et al.*, 2007). Common drying method includes sun-drying, drum, evaporation, spray, freeze-drying, vacuum thermal-drying and oven-drying. Oven-drying (Kaehler and Kennish, 1996; Robledo and Pelegrin, 1997) and freeze-drying (Norziah and Ching, 2000; Suzuki *et al.*, 1996) are two most greatly applied procedures to preserve verities of vegetable- and animal-based foodstuffs (Shadung *et al.*, 2012). Freeze drying, also known as lyophilisation, is a drying process implicating freezing of the material followed by reducing the surrounding pressure to allow the direct sublimation of the frozen water in the material from the solid to the gas phase. This method is an efficient method to extend the shelf life of foods and suitable for heat sensitive, volatile and perishable components as it employs low processing temperature (Febriyenti *et al.*, 2014).

Low-temperature storage (2–4˚C) inhibits spoilage, especially in long-term storage (Matsuura-endo *et al.*, 2006). During storage, AAs quality can change chemically and physically. Decreasing of nutritional value can occur during storage. AAs are packed to reduce the loss of quality. Light containers are intended to protect from damage, preserving, as the identity of the product and facilitate the shipping and storage. As preservation, light containers have to protect the product from external influences that could cause quality deterioration from moisture, oxygen, light, flavour, odour and chemical (Dixon, 2011). All of the AAs can undergo changes during storage. Different light containers are expected to decrease the nutritional values of AAs during storage. The types of light container used for AAs of molasses include: Metallized polypropylene container and Normal polypropylene container (Wijayanti *et al.*, 2016; Yassoralipour *et al.*, 2013).

Molasses contain valuable AAs as bioactive agents for other segments of the food industry and different industrial sectors (mainly related to health and care). AAs content in molasses can be used as raw material to obtain higher value-added products. In this study, we have attempted to clarify the following three points: First, we investigated the effects of storage temperature and light on the contents of free amino acids in sugar beet and sugar cane molasses after freeze drying. We chose two storage temperatures: 4˚C (refrigerator condition) and 25˚C (room condition). We attempted to determine at which temperature the contents of AAs in molasses decrease. Second, we investigated two differences light: Metallized polypropylene container (the darkness) and Normal polypropylene container (lighting). We attempted to determine at which light container the contents of AAs in molasses change. Third, we aim to determine the effects of the type of light and temperatures to changes in AAs content of molasses during storage to determine suitable condition for the storage of AAs.

Materials and Methods

−*Chemicals, raw materials and standard substances*

The sugar beet and sugar cane molasses with 18-25 wt% water content were obtained from Hegmatan Co., Ltd. (Hamedan, Iran) and Developed Sugar Cane Co., Ltd. (Ahvaz, Iran) respectively. Hydrochloride acid, sodium hydroxide, sodium acetate, sodium borate, HPLC-grade acetonitrile, sodium hydroxide, carbonate buffers, methanol and anhydrous sodium sulphate were obtained from Merck (Darmstadt, Germany), FMOC-Cl, ADAM and amino acid standards (Aspartic acid, Glutamic acid, Lysine, Alanine) were purchased from

Sigma(Milano, Italy). All standard solutions were stored at 4℃ and protected from light.

- *Sample preparation*

At the beginning, AAs of molasses were extracted by carbon dioxide supercritical fluid extraction (SFE). A Separex (Champigneulles, France) system in SFE mode was employed for all experiments. The extractions were accomplished using a 100 mL volume stainless steel extraction vessel. An adjustable separator (240 mL) from Separex Co. (Champigneulles, France; Varaee *et al.*, 2019) was used. The extracted solution was un-purified and it was contained as mixture of sugars and AAs. The extracted solution was filtrated with the dead end filtration system (Grib *et al.*, 2000). The polyamide Nanofilration membrane, with a total filtration area of 50 cm^2, was made by Spero Company (USA). It has molecular weight cut of 150-300 Dalton and is used at zero point charge (ZPC) in the range of pH 2-11. Extracted AAs were filtrated in pressure of 3 bar, pH of 9.5 and temperature of 47 ˚C.

- *Drying Methods*

Purified AAs has been dried by freeze drier. (Labconco, Kansas City, MO, USA) equipped with a pump (Edwards, Sanborn, NY, USA). Freeze dried samples (AAs) were first frozen to − 80 °C and then rapidly placed in a Labconco rotary sample with the condenser maintained at − 90 °C. Heat was not applied, and organic solvents or salts were not added, and the samples remained frozen under strong vacuum during the drying by means of a − 90 °C condenser and thus were lyophilized, i.e. freeze dried. The schematic of freeze dried AAs samples was shown in Figure 1.

−*Storage of the samples*

Storage of the samples at two temperatures (4˚C and 25˚C) and two different lights, Metallized polypropylene container (the darkness) and Normal polypropylene container (lighting) after 30 days was accomplished.

Fig. 1. The schematic of freeze dried AAs of sugar beet (left) and sugar cane (right) molasses

- *Derivatization procedure*

AAs were derivatized (FMOC-AA) at room temperature using a pre-column procedure. Under these conditions a volume of 300 µL of molasses or extracted AAs molasses (or a standard solution of AAs) was added with 600 µL of a 200 mM borate buffer (pH 10.0). Then, 600 µL of 15mM FMOCCl (in acetonitrile) was added to the extracted molasses and derivatization occurred. The reaction was stopped after 5 minutes by the addition of 600 µL of 300 mM ADAM (water-acetonitrile, 1:1, v/v), and the reaction lasted for 1 min to form the FMOC-ADAM complex. The sample was then filtered through a 0.45-µm polytetrafluorethylene (PTFE) and analyzed by HPLC-UV in the wavelength of 263 nm. The total time required for the derivatization procedure was 6 min (Fabiani *et al*., 2002).

- *HPLC analysis*

The analysis of the AAs in extracts was performed by high performance liquid chromatography. The HPLC system consisted of a Spectra Physics (San Jose, CA) that was equipped with a 8700 XR ternary pump, a 20-µL Rheodyne (Cotati, CA) injection loop, an SP8792 column heater, a 8440 XR UV-Vis detector that was set at 263 nm and a 4290 integrator linked via Labent to a computer. Chromatographic data were analyzed using ChromanaCH software, version 3.6.4 (Tehran, Iran). For separation, a 250- × 4.6 mm column packed with 5-µm particle size C_{18} (Sugelabor, Madrid, Spain) was employed at 25°C. A mixture of sodium acetate 50 Mm (pH = 4.2) and acetonitrile (60:40) at a flow rate of 1.0 mL min^{-1} was implemented as the mobile phase where the former and the latter were used as eluent A and B respectively. All the chromatographic measurements were carried out in the linear range (Fabiani *et al*., 2002).

Results and Discussion

In the present study, storage stability of the extraction and purification of AAs from sugar beet and sugar cane molasses was determined. There are considerable variables that can influence the shelf life efficiency of AAs. As mentioned earlier, these include temperature and light. The results have been considered in the following segments.

- *HPLC analysis of raw materials*

It should be noted that sugar beet and sugar cane molasses consist of mixtures of sugars (53 and 64% w/w), non-sugar materials AAs (19 and 10% w/w), water (16.5 and 20% w/w) and ash (11.5 and 8% w/w), respectively (Filipčev *et al*., 2016). Sugar beet and sugar cane molasses contain nearly eighteen AAs (Mee and Brooks, 1979; Rearik and Mckey, 1996; Khan *et al*., 2006). However, only four of them, aspartic acid, glutamic acid, alanine and lysine have been selected for this research study. Aspartic and glutamic acids were selected due to the fact that they are the predominant amino acids in the sugar beet and sugar cane molasses (Rearik and Mckey, 1996) and both are the most numerous neurotransmitter in the central nervous system (Alexander, 2009; Hubbord and Binder, 2016). Although, alanine and lysine are the trivial AAs in the sugar beet and sugar cane molasses (Rearik and Mckey, 1996), alanine has a considerable role in transferring nitrogen from tissues to the liver and cooperates in the metabolization of glucose for energy that leads to the balance of glucose and nitrogen in the body. Lysine is an essential AA and cannot be synthesized by mammals that are very sensitive and easily damaged (York, 2017; Bhagavan and Ha, 2011). Samples consisting of 20 µL of the diverse sugar beet and sugar cane molasses were derivatized utilizing the FMOC procedure and analyzed by HPLC as control samples to determine the four aforementioned AAs. The identification of AAs in the samples was based on the comparison between the relative retention

times of the AAs extracts with standards. The HPLC results and chromatograms of the purified sugar beet and sugar cane molasses are shown in Table 1, Figure (2a-b).

Table 1. AAs content of extracted and purified sugar beet and sugar cane molasses (mg/kg)

	Aspartic acid	Glutamic acid	Alanine	Lysine
Raw material (mg/kg)				
Sugar beet molasses	100	99.5	117	84
Sugar cane molasses	99	111	112	80

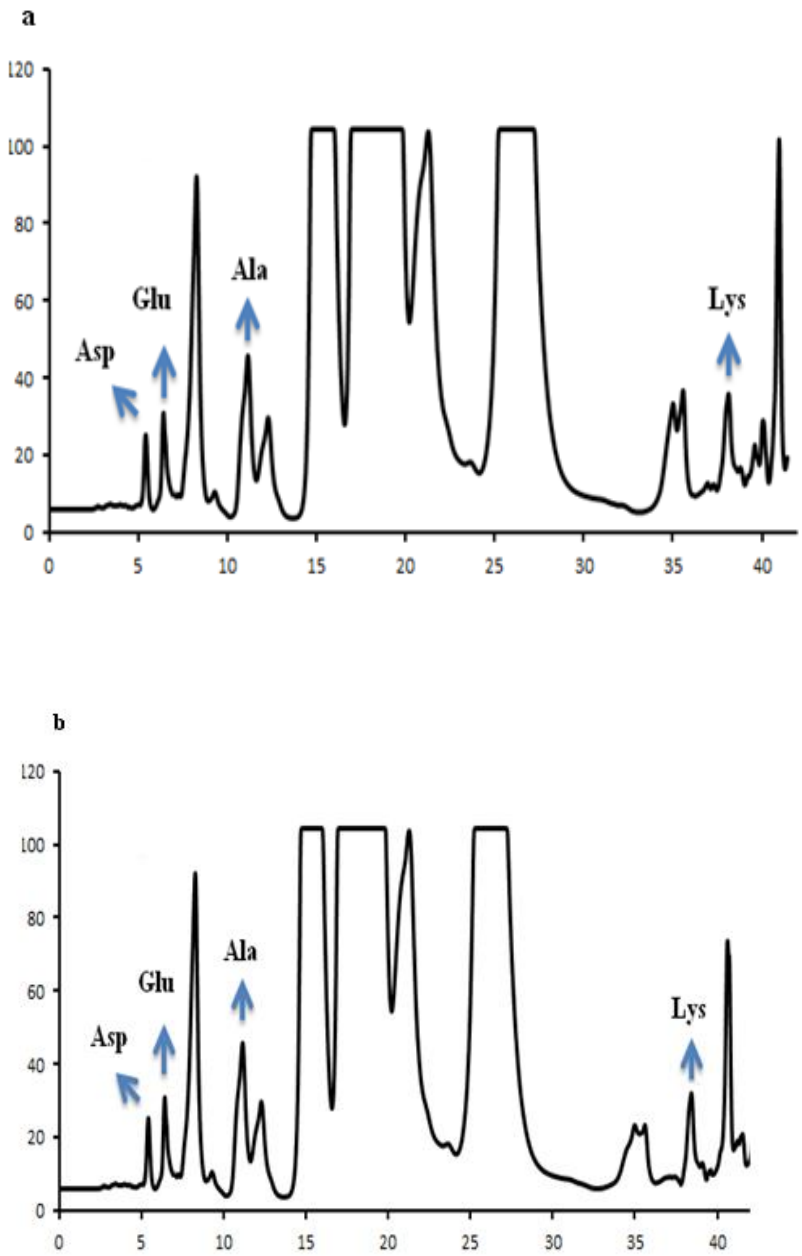

Fig. 2. The HPLC-UV chromatograms of the purified for sugar beet (a) and sugar cane (b) molasses.

- *Evaluation the shelf life of Lysine in sugar beet and sugar cane molasses after 30 days*

The results of the shelf life of lysine in both molasses at two temperatures (4˚C and 25˚C) and two different lights (Metallized polypropylene container and Normal polypropylene container) after 30 days have been discussed in the following segments. The results show that lysine is more sensitive amino acid in comparison with others therefore the rate of decline was higher than other AAs. The results are presented in Table 2 and Figure 3. Han *et al.* (2017) investigated short-term stabilities of 21 amino acids in dried blood spots in the same condition and reported nearly the similar results.

The amount of lysine was 84 and 80 (mg/Kg) in sugar beet and sugar cane molasses respectively, after storage at 4˚C and in Metallized polypropylene container declined to 77 and 74 (mg/Kg) respectively. In fact, Lysine in both molasses at 4˚C and in Metallized polypropylene container has decreased approximately 8% while, lysine in sugar beet and sugar cane molasses at 4˚C and Normal polypropylene container has diminished nearly 12%. Golbahar *et al.* (2014) evaluated the short-time stability of AAs in the dried blood spots. Their results are partly in agreement with our results.

Moreover, according to Table 2 the amount of lysine in both molasses at 25˚C

Table 2. Evaluation of the shelf life of lysine in sugar beet and sugar cane molasses at temperatures (4, 25 ˚C) and two lights (Metallized polypropylene container and Normal polypropylene container; mg/kg)

	4 ˚C , Foil	4 ˚C, Nylon	25˚C, Foil	25 ˚C, Nylon
Molasses				
Sugar beet	77	74	71	55
Sugar cane	74	70	68	52

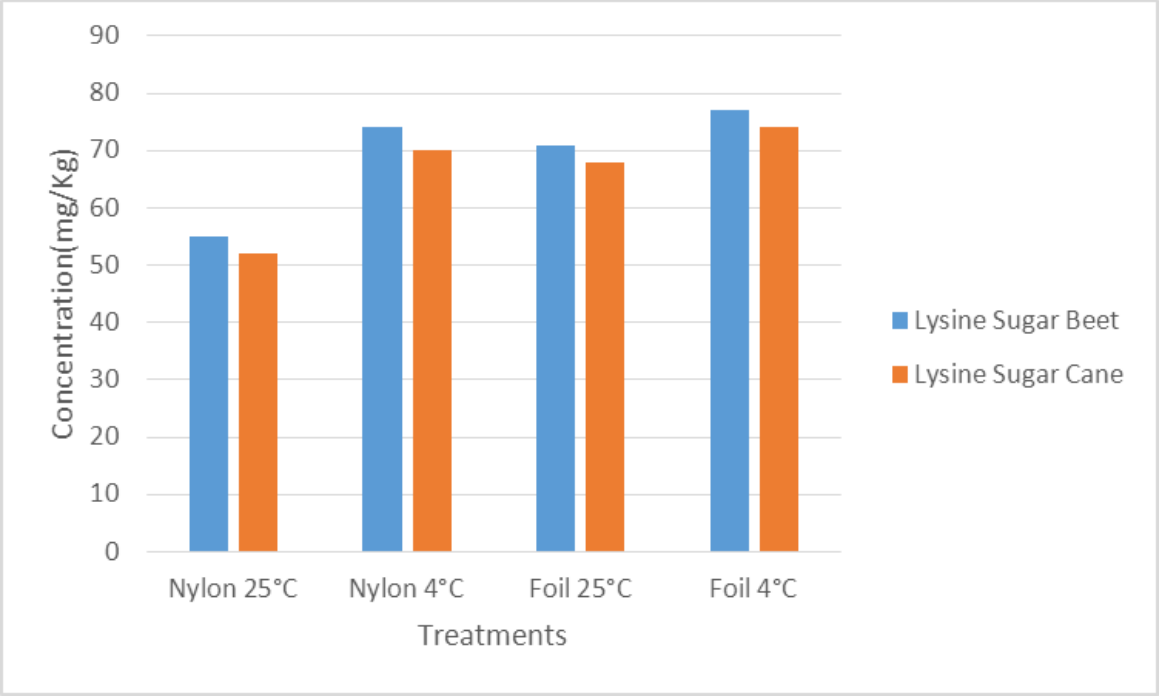

Fig. 3. Evaluation the shelf life of lysine in sugar beet and sugar cane molasses at temperatures (4, 25 ˚C) and two lights (Metallized polypropylene container and Normal polypropylene container; mg/kg).

has decreased 15 and 35% in Metallized polypropylene container and Normal polypropylene container respectively. The results was in agreement with the results of Strnadova *et al.* (2007) which studied long-term stability of amino acids and acylcarnitines in dried blood spots in different packaging.

- *Evaluation of the shelf life of Glutamic and Aspartic acid in sugar beet and sugar cane molasses after 30 days*

The consequences of the shelf life of glutamic and aspartic acids in both molasses at two temperatures (4˚C and 25˚C) and two different lights: Metallized polypropylene container and Normal polypropylene container after 30 days have been described in the following sentences and presented in Tables 3 and 4 and Figures 4 and 5.

Table 3. Evaluation of the shelf life of glutamic acid in sugar beet and sugar cane molasses at temperatures (4, 25 ˚C) and two lights (Metallized polypropylene container and Normal polypropylene container; mg/kg)

	4 ˚C , Foil	4 ˚C, Nylon	25˚C, Foil	25 ˚C, Nylon
Molasses				
Sugar beet	99.5	97.5	93	90
Sugar cane	110.5	109	103	100

Table 4. Evaluation of the shelf life of aspartic acid in sugar beet and sugar cane molasses at temperatures (4, 25 ˚C) and two lights (Metallized polypropylene container and Normal polypropylene container; mg/kg)

	4 ˚C , Foil	4 ˚C, Nylon	25˚C, Foil	25 ˚C, Nylon
Molasses				
Sugar beet	99.6	98	93	90
Sugar cane	98.5	97	92	89

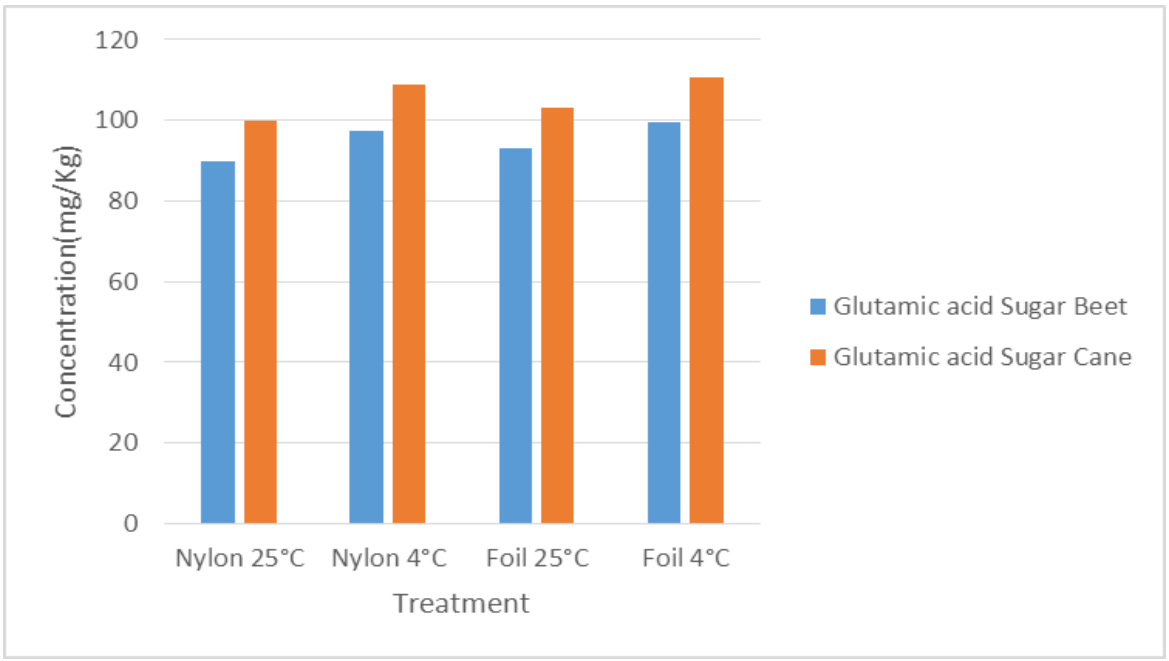

Fig. 4. Evaluation the shelf life of glutamic acid in sugar beet and sugar cane molasses at temperatures (4, 25 ˚C) and two lights (Metallized polypropylene container and Normal polypropylene container; mg/kg).

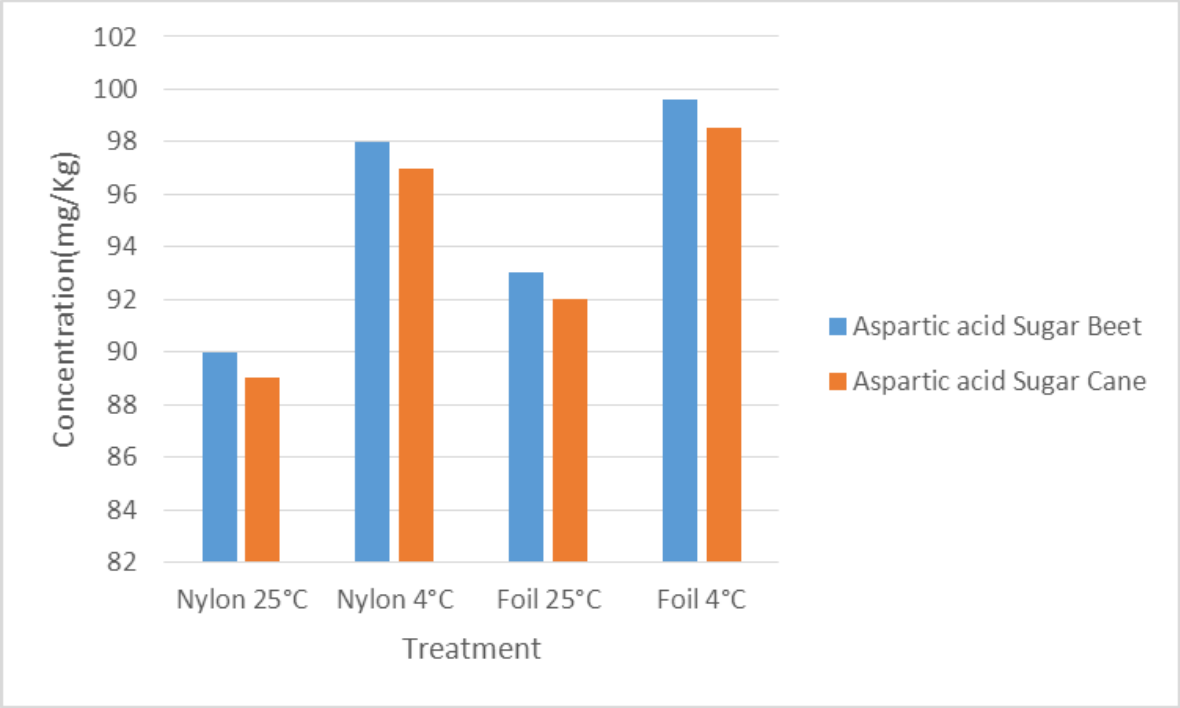

Fig. 5. Evaluation the shelf life of aspartic acid in sugar beet and sugar cane molasses at temperatures (4, 25 ˚C) and two lights (Metallized polypropylene container and Normal polypropylene container; mg/kg).

The result in Tables 3, 4 show that glutamic and aspartic acids were stable at 4˚C (refrigeration temperature) and Metallized polypropylene container. The amounts of glutamic and aspartic acids in both molasses at 4˚C and Metallized polypropylene container remained stable. Matuura-Endo *et al.* (2006) investigated the effect of storage temperature on the contents of sugars and free AAs in potato chips and the results are in agreement with our findings.

Glutamic and aspartic acids in both molasses at 4˚C and Normal polypropylene container have decreased about 2%. Han *et al.* (2017) reported approximately the same results.

According to Tables 3, 4 the amounts of both amino acids at 25˚C have declined 7 and 10% in Metallized polypropylene container and Normal polypropylene container respectively. Golbahar et al. (2014) results are nearly in agreement with our results.

- Evaluation of the shelf life of Alanine in sugar beet and sugar cane molasses after 30 days

The results of the shelf life of alanine in both molasses at two temperatures (4˚C and 25˚C) and two different lights: Metallized polypropylene container and Normal polypropylene container after 30 days are presented in Table 5 and Figure 6 that indicate alanine is more resistance AAs in comparison with other AAs, therefore the results show that it has the least reduction.

According to Table 5 and Figure 6, the amounts of alanine in both molasses at 4, 25˚C and Metallized polypropylene container remained constant while it declined around 1% at 4, 25˚C temperature and Normal polypropylene container. The finding was in agreement with the results of Han *et al.* (2017).

Conclusion

In the present study, for the first time the effect of storage on extracted AAs was successfully investigated for sugar beet and

sugar cane molasses. The evaluation of the results indicated that the optimum condition to maintain AAs is to avoid excess light in the Metallized polypropylene container since AAs might be denatured and destroyed by the light. In addition, the evaluation of the results indicated that low temperature has better effects on AAs during storage since the stability of AAs might be kept for long time at low temperature. Among the four AAs that were investigated lysine was the most sensitive and alanine was the most resistance AAs. Finally, it is worth to mention that evaluation of shelf life of AAs for sugar beet and sugar cane molasses not only improves the value added in sugar industry but also the finding in this study might be employed in other industries such as medical and pharmaceutics industries to store the valuable AAs, especially AAs that are extracted from wastes.

References

Alexander, S. P. H. (2009). Glutamate, in: *Encyclopedia of Neuroscience*, edited by Squire, L. R., Academic press, Cambridge Massachusetts, pp. 885-894

Arnáiz, E., Bernal, J., Martín, M. T., Nozal, M. J., Bernal, J. L. & Toribio, L. (2012). Supercritical fluid extraction of free amino acids from broccoli leaves. *Journal of Chromatography A*, 1250, 49-53.

Bernal, M., Ruiz, M. O., Geanta, R. M., Benito, J. M. & Escudero, I. (2016). Colour removal from beet molasses by ultrafiltration with activated charcoal. *Chemical Engineering Journal*, 283, 313–322.

Table 5. Evaluation of the shelf life of alanine in sugar beet and sugar cane molasses at temperatures (4, 25 ˚C) and two lights (Metallized polypropylene container and Normal polypropylene container; mg/kg)

	4 ˚C , Foil	4 ˚C, Nylon	25˚C, Foil	25 ˚C, Nylon
Molasses				
Sugar beet	116.8	115.8	116.4	115.5
Sugar cane	111.6	110.5	111.3	110.3

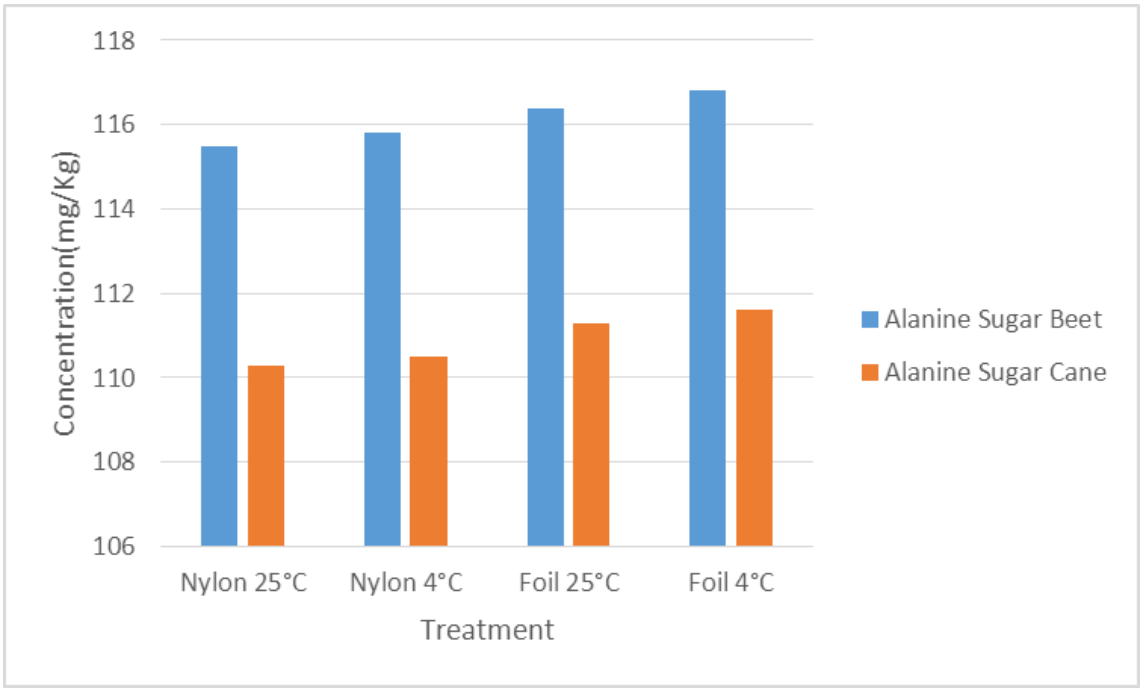

Fig. 6. Evaluation the shelf life of alanine in sugar beet and sugar cane molasses at temperatures (4, 25 ˚C) and two lights (Metallized polypropylene container and Normal polypropylene container; mg/kg).

Bernal, J. L., Nozal, M. J., Toribio, L., Diego, C., Mayo, R. & Maestre, R. (2008). Use of supercritical fluid extraction and gas chromatography–mass spectrometry to obtain amino acid profiles from several genetically modified varieties of maize and soybean. Journal of *Chromatography A*, 1192, 266-272.

Bhagavan, N. V. & Ha, C. E. (2011). Amino acids, in*: Essentials of Medical Biochemistry: with clinical cases* edited by Bhagavan, N.V., Ha, C. E., Elsevier, (pp. 19-27).

Dixon, J. (2011). Packaging Material: 9. Multilayer Packaging for Food and Beverages. ILSI Europe Packaging Materials Task Force, 48.

Dufresne, J., Hoang, T., Ajambo, J., Florentinus-Mefailoski, A. & Bowden, P. (2017). Freeze-dried plasma proteins are stable at room temperature for at least 1 year. *Clinical Proteomics*, 14, 35.

Fabiani, A., Versari, A., Parpinello, G. P., Castellari, M. & Galassi, S. (2002). High-Performance Liquid Chromatographic Analysis of Free Amino Acids in Fruit Juices Using Derivatization with 9-Fluorenylmethyl-Chloroformate. *Journal of Chromatographic Science*, 40, 14-18.

Febriyenti, F., Mohamed, N., Hamdan, M. R., Md Salleh, S. N. & Baie, S. B. B. (2014). Effect of freeze drying and spray drying processes to amino acids and fatty acids contents in Haruan (*Channa Striatus*) extract. *International Journal of Drug Delivery*, 6, 301-304.

Filipčev, B., Mišan, A., Šarić, B. & Šimurina, O. (2016). Sugar beet molasses as an ingredient to enhance the nutritional and functional properties of gluten-free cookies. *International Journal of Food Sciences and Nutrition*, 67, 249-256.

Fombong, F. T., Borght, M. V. D. & Broeck, J. V. (2017). Influence of Freeze-Drying and Oven-Drying Post Blanching on the Nutrient Composition of the Edible Insect. *Ruspolia differens*. *Insects*, 8(3), 102.

Golbahar, J., Altayab D. D. & Carreon, E. (2014). Short-Term Stability of Amino acids and Acylcarnitines in the Dried Blood Spots Used to Screen Newborns for Metabolic Disorders. *Journal of Medical Screening*, 21(1), 5–9.

Grib, H., Persin, M., Gavach, C., Piron, D. L., Sandeaux, J. & Mameri, N. (2000). Amino acid retention with alumina g nanofiltration membrane. *Journal of Membrane Science*, 172, 9–17.

Han, J., Higgins, R., Lim, M. D., Lin, K., Yang, J. & Borchers, C. H. (2017). Short-term stabilities of 21 amino acids in dried blood spots. *Clinical Chemistry*, 64, 2.

Hassan, S. W., Umar, R. A., Maishanu, H. M., Matazu, I. K., Faruk, U. Z. & Sani, A. A. (2007). The effect of drying methods on the nutrient and non-nutrients composition of leaves of Gynandropis gynandra (*Capparaceae*). *Asian Journal Biochemistry*, 2, 349-353.

Honma, T., Kaneko, A., Ohba, H. & Ohyama, T. (2012). Effect of application of molasses to paddy soil on the concentration of cadmium and arsenic in rice grain. *Soil Science and Plant Nutrition*, 58(2), 255-260.

Hubbard, A. J., Binder, D. K. (2016). Glutamate metabolism, in: *Astrocytes and Epilepsy* edited by Hubbard, A. J., Binder, D. K., Academic press, Cambridge Massachusetts, (pp. 197-224).

Kaehler, S. & Kennish, R. (1996). Summer and winter comparisons in the nutrition value of marine marcoalgae from Hong Kong. *Botanica Marina*, 39, 11-17.

Khan, S. H., Rasool, G. & Nadeemm, S. (2006). Bioconversion of Cane Molasses into Amino Acids. *Pakistan Journal of Agricultural Sciences*, 43, 157-161.

Martin-Orue, C., Bouhallab, S. & Garem, A. (1998). Nanofltration of amino acid and peptide solutions: mechanisms of separation. *Journal of Membrane Science*, 142, 225-233.

Matsuura-endo, C., Ohara-takada, A., Chuda, Y., Ono, H., Yada, H., Yoshida, M.,

Kobayashi, A., Tsuda, S., Takigawa, S., Noda, T., Yamauchi, H. & Mori, M. (2006). Effects of Storage Temperature on the Contents of Sugars and Free Amino Acids in Tubers from Different Potato Cultivars and Acrylamide in Chips. *Bioscience Biotechnology Biochemistry*, 70 (5), 1173–1180.

Mee, J. M. L., Brooks, C. C. & Stanley, R. W. (1979). Amino Acid and Fatty Acid Composition of Cane Molasses, J. Sci. Food Agric., 30, 429-432.

Rearik, D. E. & Mckey, C. (1996). The behavior of amino acids in chromatographic molasses desugarization. Conference on sugar process research New Orleans, LA, http://www.arifractal.com/images/files/The-behavior-of-amino-acids-in-chromatographic-molasses-desugarization-systems.pdf (accessed 15june 2018).

Robledo, D. & Pelegrin, Y. F. (1997). Chemical and mineral composition of six potentially edible seaweed species of Yucatán. *Botanica Marina*, 40, 301-306.

Norziah, M. H. & Ching, C. Y. (2000). Nutritional composition of edible seaweed *Gracilaria changgi*. *Food Chemistry*. 68, 69-76.

Strnadova, K. A., Holub, M., Muhl, A., Heinze, G., Ratschmann, R. & Mascher, H. (2007). Long-term stability of amino acids and acylcarnitines in dried blood spots. *Clinical Chemistry*, 53, 717-722.

Saric, L. C., Filipcev, B. V., Simurina, O. D., Plavsic, D. V., Saric, B. M., Lazarevic, J. M. & Milovanovic, I. L. (2016). Sugar beet molasses: properties and applications in osmotic dehydration of fruits and vegetables, *Food and Feed Research*, 43, 135-144.

Samavat, S. & Samavat, S. (2014). The effects of folic acid and sugar cane molasses on yield and qualities of tomato. *International Research Journal of Applied and Basic Sciences*, 8(3), 266-268.

Shadung, K. G., Mphosi, M. S. & Mashela, P. W. (2012). Influence of drying method and location on amino acids and mineral elements of Sternocera orissa Buguet 1836 (*Coleoptera: Buprestidae*) in South Africa. *African Journal of Agricultural Research*, 7(46), 6130-6135.

Suzuki, T., Ohsugi, Y., Yoshie, Y., Shirai, T. & Hirano, T. (1996). Dietary fiber content, water holding capacity and binding capacity of seaweeds. *Fish Science*, 62, 454-461.

Valli, V., Gomez-Caravaca, A. M., Caboni, M. F., Bordoni, A., Nunzio, M. D. & Danesi, F. (2012). Sugar cane and Sugar beet molasses, antioxidant-rich alternatives to refined sugar. *Journal Agriculture Food Chemistry*, 60, 12508–12515.

Varaeea, M., Honarvar, M., Eikani, M. H., Omidkhah, M. R. & Moraki, N. (2019). Supercritical fluid extraction of free amino acids from sugar beet and sugar cane molasses. *The Journal of Supercritical Fluids*, 144, 48–55.

Wang, G., Zhang, C., Sun, M., Zhang, X., Wu, C. & Wu, Y. (2017). Separation of mixed amino acids by BMED process using porous SPES and SPSf cation exchange membranes. *Separation and Purification Technology*, doi: http://dx.doi.org/10.1016/j.seppur.2017.07.020.

Wijayanti, I., Surti, T., Anggo, A. D. & Susanto, E. (2016). Effect Different Packaging on Proximate and Lysine Content of Milkfish [Chanos chanos (Forsskål, 1775)] Floss During Storage. *Aquatic Procedia*, 7, 118 – 124

Yassoralipour, A., Bakar, J., Abdul Rahman, R., Abu Bakar, F. & Golkhandan, E. (2013). Effect of Different Temperatures on the Free Amino Acids, Physico-Chemical and Microbial Changes during Storage of Barramundi (*Lates calcarifer*) Fillets. *Advance Journal of Food Science and Technology*, 5(7), 822-828.

York, M. J. (2017). Clinical Pathology, in: *A Comprehensive Guide to Toxicology in Nonclinical Drug Development (Second Edition)* edited by Faqi, A. S., Academic

press, Cambridge Massachusetts, (pp. 325-374).

Zhu, G., Zhu, X., Xiao, Z., Zhou, Z., Feng, N. & Niu, Y. (2015). A review of amino acids extraction from animal waste biomass and reducing sugars extraction from plant waste biomass by a clean method. *Biomass Conversion and Biorefinery*, 5 (3), 309–320.