International Food Research Journal 21(6): 2363-2367 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

Physicochemical and antioxidant studies on oven-dried, freeze-dried and spray-dried agaro-oligosaccharide powders

Kang, O. L., Yong, P. F., *Ma'aruf, A. G., Osman, H. and Nazaruddin, R.

Faculty Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

Article history

Received: 27 June 2013
Received in revised form: 8 April 2014
Accepted: 10 April 2014

Keywords

Agaro-oligosaccharide powders
Physicochemical properties
Antioxidant activities

Abstract

In this work, oven-dried, freeze-dried and spray-dried agaro-oligosaccharide powders were characterized to investigate their physicochemical and antioxidant properties. Agaro-oligosaccharide powders were shown to exhibit high water solubility index (88.73 – 95.88%), water absorption capacity (0.96 – 2.57 g/g) and oil absorption capacity (0.40 – 0.45 g/g). Agaro-oligosaccharide powders were shown to possess moderate DPPH radical scavenging activity (10.65 – 14.59%), ABTS radical scavenging activity (44 .47 – 65.61%) and ferric reducing antioxidant activity (0.165 – 0.353). Agaro-oligosaccharide powders were further characterized with respect to thermal and pH stability. Agaro-oligosaccharide powders were shown to exhibit high temperature resistance (≤ 100°C) and acid/alkaline resistance.

Introduction

Galactan oligosaccharides have attracted substantial research interest in the past decades (Chen *et al*., 2005). Galactan oligosaccharides are well known dietary antioxidants (Yuan *et al*., 2005). Several oligosaccharides have thus developed into functional food ingredients and nutritional supplements (Barreteau *et al*., 2006, Qiang *et al*., 2009). Up to date, however, limited reports are available in the literature on agaro-oligosaccharide production. Agarose, a purified linear galactan polysaccharide isolated from marine algae, is the most preferred to agaro-oligosaccharide production (Chen *et al*., 2005; Saraswathi *et al*., 2011). Agarose is connected in a chain through α-(1-3) and β-(1-4) glycosidic bonds (Michel *et al.*, 2006). Agarose is cleaved at α-(1-3) glycosidic bonds into agaro-oligosaccharide either through acid hydrolysis or enzymatic degradation (Michel *et al.*, 2006, Saraswathi *et al.*, 2011).

Operational conditions can exert significant influence on oligosaccharide characteristics (Grabowski *et al*., 2006, Gulia *et al*., 2010). Drying operations is often considered the most important stage in dried powder productions, since it affects color and appearance as well as functionalities (Gulia *et al*., 2010, Suvarnakuta *et al*., 2011). Several drying (e.g. air drying, drums drying, freeze drying and spray drying) technologies are available in dried powder production (Phisut *et al.*, 2012). Each drying method has its own unique advantages and limitations; therefore, an appropriate drying technique must be carefully chosen.

Spray drying is the most extended technique; it is rather simple and scalable method in dried powder production (Kha *et al.*, 2010). Spray drying is applicable to heat sensitive, thermoplastic and/or hygroscopic materials. Spray drying can give rise to dried powders, granules, or agglomerates at competitive requirement (Grabowski *et al.*, 2006). In this research, three different drying (i.e., oven drying, freeze drying and spray drying) techniques were implemented in dried agaro-oligosaccharide production. Physicochemical analysis and antioxidant assay were conducted in dried agaro-oligosaccharide powders.

Materials and Methods

Materials

Agarose was purchased from Sigma-Aldrich Co. (St. Louis, USA). Celluclast 1.5L® (cellulase from Trichoderma reesei) was purchased from Novozymes A/S (Bagsvaerd, Denmark). Celluclast 1.5L had 700 EGU/g cellulase activities.

Hydrolysis reaction

In this experiment, agarose degradation was conducted using shake flask method. The hydrolysis reaction was conducted at a selected solid to liquid ratio (1: 100) and 5.0 wt. % enzyme loads. The reaction mixture was incubated at constant temperature (50 ± 2°C) for 24 hours. The reaction mixture was centrifuged, and the oligosaccharide hydrolysate was then dried using three different methods: oven drying, freeze drying and spray drying.

*Corresponding author.
Email: *magsutton@yahoo.com*

Drying process

Oven drying

Oligosaccharide hydrolysate was oven-dried in a vacuum oven (Venticell, MMM, Einrichtungen, Germany) at 60°C until constant weight (24 hours). The dried oligosaccharide powders were sealed airtight and stored at 5°C until analyses.

Freeze drying

Oligosaccharide hydrolysate was frozen in refrigerator at 4°C and then freeze-dried in a freeze dryer (Freezone Plus 6, Labconco, USA) at -40°C and 0.3 mPa until constant weight (72 hours). The dried oligosaccharide powders were sealed airtight and stored at 5°C until analyses.

Spray drying

Oligosaccharide hydrolysate was spray-dried in a pilot scale spray dryer (Büchi 190, Switzerland) at constant flow rate 15ml/min and inlet and outlet temperature 180 and 80°C. The dried oligosaccharide powders were sealed airtight and stored at 5°C until analyses.

Physicochemical characterization

Water solubility index (WSI)

Water solubility index (WSI) was determined using shake-flask method. 0.5 g sample was suspended in 50 ml distilled water. The suspension was incubated at 60°C for 1 hour. The suspension was centrifuged at 4,000 rpm for 30 min, and the insoluble residue was recovered and dried at 60°C until constant weight. Water solubility index (%) was calculated using following formula:

$$\text{Water solubility index (\%)} = \frac{\text{Insoluble residue weight}}{\text{Original sample weight}} \times 100$$

Water absorption capacity (WAC)

Water absorption capacity (WAC) was determined using centrifugal method. 0.1 g sample was suspended in 10ml distilled water. The suspension was incubated at room temperature for 30 min. The suspension was centrifuged at 4,000 rpm for 30 min, and the supernatant was collected and measured in a graduated cylinder (10 ml). Water absorption capacity (g/g) was calculated using following formula:

$$\text{WAC (g/g)} = \frac{(\text{Initial solution volume} - \text{Final solution volume}) \times \text{Water density}}{\text{Original sample weight}}$$

Oil absorption capacity (OAC)

Oil absorption capacity (OAC) was determined using centrifugal method. 0.1 g sample was suspended in 10ml oil (density = 0.92 g/ml). The suspension was incubated at room temperature for 30 min. The suspension was centrifuged at 4,000 rpm for 30 min, and the supernatant was collected and measured in a graduated cylinder (10 ml). Oil absorption capacity (g/g) was calculated using following formula:

$$\text{WOC (g/g)} = \frac{(\text{Initial solution volume} - \text{Final solution volume}) \times \text{Oil density}}{\text{Original sample weight}}$$

Color parameter

Color parameters were determined using colorimeter (Minolta Chroma meter CR-300, USA). Sample was poured into a clear glass petri dish and color coordinate values (lightness, L^*, redness, a^*, and yellowness, b^*) were recorded.

Antioxidant characterization

1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay

DPPH radical scavenging activity was determined using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay (Yang *et al*., 2006, Kong *et al*., 2010). 0.5 ml oligosaccharide sample (10 mg/ml, in distilled water) was added to 2.5 ml DPPH reagent (0.2 mM). The reaction mixture was kept in dark at ambient temperature for 30 min. The absorbance was measured using a spectrophotometer at 517 nm. DPPH radical scavenging activity (%) was calculated using following formula:

$$\text{DPPH radical scavenging activity (\%)} = 1 - \frac{\text{Abs}_{515}\ \text{sample}}{\text{Abs}_{515}\ \text{DPPH solution}} \times 100$$

2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay

ABTS radical scavenging activity was determined using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay (Yang *et al.*, 2006, Kong *et al*., 2010). 0.5 ml oligosaccharide sample (10 mg/ml, in distilled water) was added to 2.5 ml ABTS reagent. The reaction mixture was kept in dark at ambient temperature for 10 min. The absorbance was measured using a spectrophotometer at 735 nm. ABTS radical scavenging activity (%) was calculated using following formula:

$$\text{ABTS radical scavenging effect (\%)} = 1 - \frac{\text{Abs}_{735}\ \text{sample}}{\text{Abs}_{735}\ \text{ABTS solution}} \times 100$$

Ferric reducing antioxidant power (FRAP) assay

Reducing power was determined using ferric reducing antioxidant power (FRAP) assay (Barahona *et al*., 2011). 0.5 ml oligosaccharide sample (10 mg/ml, in distilled water) was added to 2.5 ml FRAP reagent (TPTZ, $FeCl_3$, acetate buffer in 10:1:1 ratio). The reaction mixture was kept in dark at ambient temperature for 10 min. The absorbance was measured using a spectrophotometer at 593 nm. Reducing power was calculated using following formula:

$$\text{Reducing power} = Abs_{593}\text{sample} - Abs_{593}\text{ FRAP reagent}$$

Results and discussions

Physicochemical properties

Three different drying (e.g. oven-drying, freeze-drying and spray-drying) methods were applied to produce agaro-oligosaccharide powders. These drying methods were significantly influence the oligosaccharide characteristics. Water solubility is the most important determinant in food product development (Ahmed *et al*., 2011). In this experiment, water solubility index were varied in the range between 88.73 – 95.88 %. The highest solubility index was observed in spray dried oligosaccharide powders ($P < 0.05$). The highest solubility index is attributed to small amorphous aggregates. In most cases, spray drying at a high inlet temperature (180°C) can give rise to more soluble amorphous structure, and thus increase the dissolution rate. Spray drying at a high atomization speed can give rise to small particle size (Souza *et al*., 2009, Tee *et al.*, 2012).

Water and oil absorption is most desirable functional properties in food systems. Water and oil absorption can affect the texture and sensoric characteristics such as aroma, taste and mouth feel (Osundahunsi *et al*., 2003, Baljeet *et al*., 2010). In this experiment, water and oil absorption capacities were varied in the range between 0.96 – 2.57 and 0.40 – 0.45 g/g. The highest absorption capacities were observed in freeze dried oligosaccharide powders ($P < 0.05$). The highest absorption capacities could attribute to porous coarse aggregates (Sanful *et al*., 2013). In most cases, freeze drying can give rise to loose porous structure, and thus absorb more water molecule. Oven drying, on the other hand, can give rise to compact structure.

Color is an important appearance attribute in food products. Color is thus critical to product acceptance (Pedreschi *et al*., 2005). Lightness (L^*), redness (a^*) and yellowness (b^*) were significantly different among the oligosaccharide samples ($P < 0.05$). The highest L^* value (74.14) and lowest a^* value (2.32) was observed in freeze-dried oligosaccharide powder. The highest L^* value could attribute to minimal color deterioration. Freeze drying can retarded oxidation and other chemical reactions, and thus minimal color deterioration (Ratti *et al.*, 2001). Meanwhile, the lowest L^* value (64.90) and highest a^* value (5.19) was observed in oven-dried oligosaccharide powder. The lowest L^* value could attribute to color degradation. Oven drying can cause oxidative degradation, and thus lead to color change (turn into darker color).

Table 1. Solubility, water and oil absorption capacity

	Solubility (%)	WAC (g/g)	OAC (g/g)
Oven dried oligosaccharide	89.94 ± 1.72	1.91 ± 0.09	0.43 ± 0.06
Freeze dried oligosaccharide	88.73 ± 1.36	2.57 ± 0.53	0.45 ± 0.01
Spray dried oligosaccharide	95.88 ± 0.44	0.96 ± 0.01	0.40 ± 0.02

Table 2. CIELAB Color parameter

	Color parameter		
	L*	a*	b*
Oven dried oligosaccharide	64.90 ± 1.16	5.19 ± 0.13	9.57 ± 0.32
Freeze dried oligosaccharide	74.14 ± 1.61	2.32 ± 0.06	9.73 ± 0.10
Spray dried oligosaccharide	67.96 ± 0.53	5.09 ± 0.40	9.72 ± 1.03

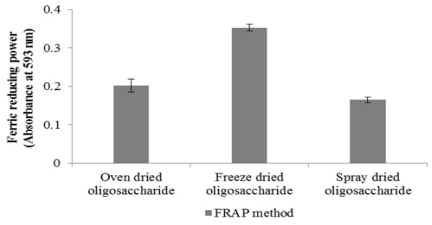

Figure 1. Ferric reducing power (Each value represents the mean percentage ± SD from three independent experiments)

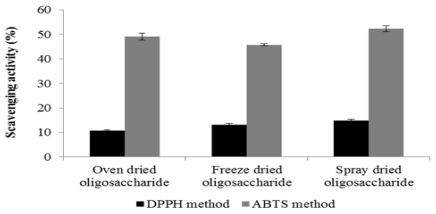

Figure 2. DPPH and ABTS scavenging activities (Each value represents the mean percentage ± SD from three independent experiments)

Antioxidant activity

Oligosaccharide powders were further characterized to investigate their antioxidant activities. In this experiment, oligosaccharide powders were effective at reducing iron (III) and scavenging radical; therefore, oligosaccharide end groups (–OH groups) are thought responsible for their antioxidant activities (Tsao, 2010). The C-2 and C-6 hydroxyls are believed able to neutralize DPPH and ABTS radical, either through a single-electron transfer reaction or through a hydrogen atom transfer reaction. Ferric reducing antioxidant power (FRAP) were found in the range between 0.165 – 0.353. FRAP values were significantly difference between oven-dried, freeze-dried and spray-dried oligosaccharide powders.

DPPH radical scavenging activities were found in the range between 10.65 – 14.59 %. DPPH radical scavenging activities were not significantly different between freeze-dried and spray-dried oligosaccharide powders, while both were significantly higher than oven-dried oligosaccharide powder ($P \leq 0.05$). Meanwhile, ABTS radical scavenging activities were found in the range between 44 .47 – 65.61%. The scavenging efficacies were significantly different ($P < 0.05$) between oven-dried, freeze-dried and spray-dried oligosaccharide powders.

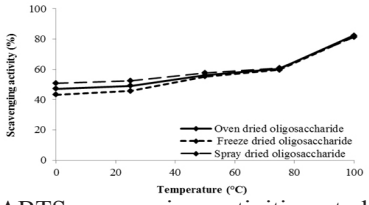

Figure 3. ABTS scavenging activities at different heat treatments (Each value represents the mean percentage ± SD from three independent experiments).

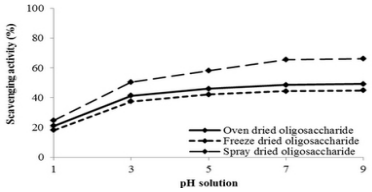

Figure 4. ABTS scavenging activities at different pH conditions (Each value represents the mean percentage ± SD from three independent experiments)

In this experiment, ABTS radical scavenging activity was consistently higher than DPPH radical scavenging activities. ABTS method is therefore considered more reliable than the DPPH method (Teow *et al.*, 2007). In most cases, ABTS radical cation ($ABTS^{\bullet+}$) is reactive towards most antioxidants, and it is applicable to determine lipophilic and hydrophilic antioxidant capacities over a wide pH range (unlike DPPH, DPPH is sensitive to acidic pH) (Cano *et al*., 2000).

Antioxidant stability

Oven-dried, freeze-dried and spray-dried agaro-oligosaccharide powders were subjected to acid and heat treatments. Oligosaccharide powders were alkaline- and heat-stable, and thus easier to incorporate into foodstuffs such as bakery product and fermented-milk product. In this experiment, scavenging activity was significantly ($p < 0.05$) enhanced through heat treatment; showed chain breaking activity was higher than those unheated oligosaccharide powder. High radical scavenging activity was observed at 100°C. High radical scavenging activity is attributed to brown pigment (reductant compound) formation throughout heating (Kusznierewicz *et al*., 2008).

Radical scavenging activity was relatively stable across a pH range from 3.0 – 9.0. High radical scavenging activity was observed at pH 7.0 – 9.0. High radical scavenging activity is attributed to hydroxyl deprotonation at alkaline pH condition. Hydroxyl deprotonation is important to facilitate electron transfer reaction: $ABTS^{+\bullet} + e \rightarrow ABTS$ (Li *et al.*, 2011).

Conclusion

Drying techniques were shown to exert significant effect on oligosaccharide characteristic. Spray drying was most suitable method to produce high quality dried oligosaccharide powders, i.e. better functional and antioxidant properties. Both pH and heat treatment were shown to exert significant influence on the antioxidant activities.

Acknowledgement

This work was financially supported by Department of Fisheries, MOA: STGL-007-2010.

References

Ahmed, S.H., Isam, A., Mohamed Ahmed, I.A., Eltayeb, M.M., Ahmed, S.O., and Babiker, E.E. 2011. Functional properties of selected legumes flour as influenced by pH. Journal of Agricultural Technology 7(5): 1291-1302.

Baljeet, S.Y., Ritika, B.Y. and Roshan, L.Y. 2010. Studies on functional properties and incorporation of buckwheat flour for biscuit making. International Food Research Journal 17: 1067-1076.

Barahona, T., Chandía, N.P., Encinas, M.V., Matsuhiro, B. and Zúñiga, E.A. 2011. Antioxidant capacity of sulfated polysaccharides from seaweeds. A kinetic approach. Food Hydrocolloids 25: 529-535.

Barreteau, H., Delattre, C. and Michaud, P. 2006. Production of oligosaccharides as promising new food additive generation. Food Technology and Biotechnology 44(3): 323-333.

Cano A., Acosta M. and Arnaro M. B. 2000. A method to measure antioxidant activity in organic media: application to lipophilic vitamins. Redox Report 5(6): 365-370.

Chen, H.M., Zheng, L. and Yan, X.J. 2005. The preparation and bioactivity research of agaro-oligosaccharide. Food Technology and Biotechnology 43(1): 29-36.

Grabowski, J.A., Truong, V.D. and Daubert, C.R. 2006. Spray-drying of amylase hydrolyzed sweet potato puree and physicochemical properties of powder. Journal of Food Science 71: 209-217.

Gulia, A., Sharma, H.K., Sarkar, B.C., Upadhyay, A. and Shitandi, A. 2010. Changes in physicochemical and functional properties during convective drying of aloe vera (*Aloe barbadensis*) leaves. Food and Bioproducts Processing 88(2-3): 161-164.

Kha, T.C., Nguyen, M.H. and Roach, P.D. 2010. Effects of spray drying conditions on the physicochemical and antioxidant properties of the Gac (*Momordica cochinchinensis*) fruit aril powder. Journal of Food Engineering 98: 385-392.

Kong, F.L., Zhang, M.W., Liao, S.T., Yu, S.J., Chi, J.W. and Wei, Z.C. 2010. Antioxidant activity of polysaccharide-enriched fractions extracted from pulp tissue of *Litchi chinensis* Sonn. Molecules: 15: 2152- 2165.

Kusznierewicz, B., Anna Smiechowska, A., Bartoszek, A. and Namiesnik, J. 2008. The effect of heating and fermenting on antioxidant properties of white cabbage.

Food Chemistry 108: 853-861.

Li, X.C., Wang, X.Z., Chen, D.F. and Chen, S.Z. 2011. Antioxidant activity and mechanism of Protocatechuic Acid *in vitro*. Functional Foods in Health and Disease 7: 232-244.

Michel, G., Nyval-Collen, P., Barbeyron, T., Czjzek, M. and Helbert, W. 2006. Bioconversion of red seaweed galactans: a focus on bacterial agarases and carrageenases. Applied Microbiology and Biotechnology 71(1):23-33.

Osundahunsi, O.F., Fagbemi, T.N., Kesselman, E. and Shimoni, E. 2003. Comparison of the physicochemical properties and pasting characteristics of flour and starch from red and white sweet potato cultivars. Journal of Agricultural and Food Chemistry 51: 2232-2236.

Pedreschi, F., Moyano, P., Kaack, K. and Granby, K. 2005. Color changes and acrylamide formation in fried potato slices. Food Research International 38: 1- 9.

Phisut, N. 2012. Spray drying technique of fruit juice powder: some factors influencing the properties of product. International Food Research Journal 19(4): 1297-1306.

Qiang, X., Chao, Y.L. and Wan, Q.B. 2009. Health benefit application of functional oligosaccharides. Carbohydrate Polymers 77: 435- 441.

Ratti, C. 2001. Hot air and freeze-drying of high-value foods: a review. Journal of Food Engineering 49: 311-219.

Saraswathi, S., Vasanthabharathi, V., Kalaiselvi, V. and Jayalakshmi, S. 2011. Characterization and optimization of agarase from an estuarine *Bacillus subtilis*. African Journal of Microbiology Research 5(19): 2960-2968.

Souza, A.S., Borges, S.V., Magalhaes, N.F., Ricardo, H.V., Cereda, M.P. and Daiuto, E.R. 2009. Influence of spray drying conditions on the physical properties of dried pulp tomato. Ciência e Tecnologia de Alimentos 29(2): 291-294.

Suvarnakuta, P., Chaweerungrat, C. and Devahastin, S. 2011. Effects of drying methods on assay and antioxidant activity of xanthones in mangosteen rind. Food Chemistry 125: 240-247.

Tee, L.H., Luqman Chuah, A., Pin, K.Y., Abdull Rashih, A. and Yusof, Y.A. 2012. Optimization of spray drying process parameters of *Piper betle* L. (Sirih) leaves extract coated with maltodextrin. Journal of Chemical and Pharmaceutical Research 4(3):1833-1841.

Tsao, R. 2010. Chemistry and biochemistry of dietary polyphenols. Nutrients 2: 1231-1246.

Teow, C.C., Truong, V.D., McFeeter, R.F., Thompson, R.L., Pecota, K.V. and Yencho, G.C. 2007. Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. Food Chemistry 103: 829-838.

Yang, B., Wang, J., Zhao, M., Liu, Y., Wang, W. and Jiang, Y. 2006. Identification of polysaccharides from pericarp tissues of litchi (*Litchi chinensis* Sonn.) fruit in relation to their antioxidant activities. Carbohydrate Research 341: 634-638.

Yuan, H.M., Zhang, W.W., Li, X.G., Lu, X.X., Li, N., Gao, X.L. and Song, J.M. 2005. Preparation and in vitro antioxidant activity of κ-carrageenan oligosaccharides and their oversulfated, acetylated, and phosphorylated derivatives. Carbohydrate Research 340: 685-692.