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Improved Astaxanthin Availability due to Drying and Rupturing of the Red Yeast, *Xanthophyllomyces dendrorhous*

Gil-Hwan An*, Jae-Yeon Song, Woong-Kwon Kwak, Bong-Duk Lee, Kyung-Bin Song, and Jae-Eul Choi

College of Agriculture and Life Science, Chungnam National University, Daejeon 305-764, Korea

Abstract To be used as a source of astaxanthin by animals, the red yeast *Xanthophyllomyces dendrorhous* needs to be dried and the cell wall ruptured. Spray-drying and flat-roller milling successfully prepared the yeast as a feed additive with little loss of astaxanthin. Spray-drying successfully dried the yeast with negligible decomposition of astaxanthin compared to drum-drying. By repeated milling with a flat-roller mill, astaxanthin extracted with ethanol increased from 0.01 to 1.31 mg astaxanthin/g yeast. This method did not decompose astaxanthin in contrast to chemical digestion of the cell wall. Flat-roller milling effectively flattened and cracked the dried cells. Astaxanthin in yeast prepared by spray-drying and flat-roller milling was well absorbed by animals. Specifically, when spray-dried and milled yeast was supplied in the feed (40 mg astaxanthin/kg feed), astaxanthin was successfully absorbed (1,500 ng/mL blood and 1,100 ng/g skin) by laying hens.

Key words: drying, cell wall, astaxanthin, *Xanthophyllomyces dendrorhous*

Introduction

Astaxanthin is not only a pigment for fish, poultry, and crustaceans (1) but also a strong antioxidant (2). *Xanthophyllomyces dendrorhous* has the following approximate composition: ash, 5.6%; carbohydrate, 40.3%; protein, 30.1%; RNA, 8.2%; lipid, 17.0% (3); and astaxanthin, 0.06-0.3% (1). Since fats and possibly carotenoids have been demonstrated to contribute to the flavor of salmonids (4), the high lipid content of *X. dendrorhous* and its content of carotenoids could improve the flavor of commercially pen-reared salmon compared to synthetic feed additives. Astaxanthin successfully accumulated in the fatty tissues when fed to chickens (5, 6), and the accumulated astaxanthin increased the quality of chicken by improving flavor, delaying oxidation, and adding pigmentation (6).

However, astaxanthin is not deposited in the flesh, eggs, or carapaces of various animals fed the intact yeast (7). When the cell wall of *X. dendrorhous* was mechanically ruptured or enzymatically digested, its pigments were transferred to rainbow trout, lobsters, and egg yolks (3, 7). However, those methods are costly and thus are not practical (8). Astaxanthin in the yeast can be made bio-available to laying hens by chloric acid treatment and spray-drying (6). However, treatment with heat and chloric acid degraded 45% of the astaxanthin (8).

Processes of cell wall degradation have, so far, been carried out before drying. Through this study, we have developed a method of rupturing the cell wall of the yeast after drying with minimized decomposition of astaxanthin. This method also can be used for astaxanthin extraction from the yeast.

Materials and Methods

Preparation of dried yeast *X. dendrorhous* strain 2A2N (9) was used in this study. The media and culture conditions used in this study were previously described (10). Plant-scale (fermentor size, 30 ton; dryer size, 5 ton) spray- and drum-dried *X. dendrorhous* was purchased from Jeil Biotech. Co. (Ansan, Korea). The inlet and outlet temperatures for the spray-dryer (Samyoung Chemical Co., Seoul, Korea) was 190 and 90°C, respectively. The drum temperature for the drum-dryer (Tanhay Pneumatic Ltd., Seoul, Korea.) was 150°C. The pilot-scale spray-dryer (spray-dry-1; Eyela Co., Tokyo, Japan) was operated at 150°C for the inlet and at 85°C for the outlet, and the inlet cell concentration was 200 g/L.

Dried yeast was observed on a Hemocytometer under an optical microscope to measure particle size. To observe yeast cell shape, a scanning electron microscope (SEM, XL30 ESEM 6805; Philips, Netherlands) was used.

Carotenoid analysis The total carotenoid content was measured after treatment with dimethyl sulfoxide (DMSO) (8). One mL each of DMSO, acetone, petroleum ether, and 20% NaCl were serially added into 0.01 g of dried yeast. After centrifugation the upper petroleum ether layer was filtered and the absorbance at 476 nm was measured (11) with a spectrophotometer (CARY 100 Conc; Varian, Inc., Cary, NC, USA). Since there were broken or modified carotenoids, total carotenoid (mg/g yeast) was calculated as an astaxanthin equivalent by measuring the absorbance at 476 nm.

To measure the degree of carotenoid degradation during drying, the extracted carotenoids from the yeast were analyzed by thin layer chromatography (TLC plate; Silica gel 60, 5×20 cm, 0.25 mm thickness; E. Merck, Darmstadt, Germany). The solvent system used for TLC was acetone-petroleum ether (20:80, v/v). The carotenoid bands were then scraped and eluted with 1 mL of acetone. Since quantification of broken carotenoids was not possible, the carotenoid content was calculated by the formula: (absorbance of each carotenoid)/(sum of absorbances of all carotenoids). Absorbances were measured at the

*Corresponding author: Tel: 82-42-821-6730; Fax: 82-42-823-4835
E-mail: ghahn@cnu.ac.kr
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wavelengths of peak maxima.

The carotenoid extracts were also analyzed by HPLC (Younglin Instrument Co., Seoul, Korea). Twenty mL of carotenoid extract was injected into a Nucleosil column (100Å) (MetaChem Technologies Inc., Torrance, CA, USA) and carotenoids were detected with a UV-visible detector at 476 nm. The mobile system was *t*-butyl methyl ether:hexane:isopropanol:methanol (30:65:2.5:2.5, v/v) and the flow rate was 1.5 mL/min.

Cell wall rupturing and carotenoid extraction The cell wall of the dried yeast was ruptured by a flat-roller mill (Gwangnong Machinery Co., Seoul, Korea). Milling conditions are 210 rpm and 4,000 J/sec. Since carotenoids in the dried yeast can be extracted by ethanol and cell wall degradation affects carotenoid extraction efficiency, ethanol extraction of the dried yeast cells was used to measure the degree of cell wall rupturing. Five mL of ethanol was added to 0.1 g of dried yeast sample while vortexing. After centrifugation, the upper ethyl alcohol layer was filtered and measured absorbance at 476 nm.

Pigmentation of laying hens by *X. dendrorhous* The conditions and chickens for the feeding trial were described previously (6, 12). One-day-old 160 male Ross broilers were randomly allotted to 16 raised wire-floor cages (100×72×50 cm). Two experimental dietary treatments with eight cages (10 birds per cage) per treatment were used in a completely randomized design. One bird per cage was randomly selected for analysis and thus 8 replicates per treatment were designed. Astaxanthin was supplied at 40 mg/kg feed in the form of milled yeast. Other general feed components have been described (6). During the feeding trial, one bird from each pen was randomly selected and blood was obtained from the wing vein. To 1 mL of EDTA-treated whole blood, 2 mL of dimethyl sulfoxide, 2 mL of acetone, 1 mL of petroleum ether, and 2 mL of 20% NaCl were serially added while vortexing. After centrifugation (1,800×g, 3 min), the upper petroleum ether layer was filtered and used for HPLC analysis. After blood sampling, these birds were slaughtered for various carcass analyses. Skin for carotenoid analysis was roughly cut with scissors and homogenized (Polytron PT-MR2100; Kinematica Co., Switzerland). Carotenoid obtained from the skin contained high contents of lipids and thus the extracts were pretreated as follows. The petroleum ether layer was loaded on a silica thin layer chromatography plate. Silica containing the carotenoid was scraped and eluted with acetone. The acetone was filtered and used for HPLC analysis. All data were subjected to one-way ANOVA. When the *F*-value was significant ($p \leq 0.05$ or $p \leq 0.01$), a post-ANOVA test was conducted by using Tukey's test.

Stability of astaxanthin during storage The astaxanthin extracts with ethanol were concentrated by blowing nitrogen gas and mixed with skim milk powder. The mixture of astaxanthin extract and skim milk powder was prepared to contain 1 mg astaxanthin/g powder. The powder mixture was kept in a refrigerator (4°C) or in an incubator (30°C) after capsulation.

Results and Discussion

Drying and milling The yeast powder prepared by plant-scale spray-drying contained a wide range of cluster sizes (8-30 μm) (Fig. 1b), whereas that prepared by lab-scale spray-drying was homogeneous (8-10 μm) (Fig. 1a). The larger size and longer flight of the droplets in plant-scale spray-drying resulted in large dried cell clusters.

For the physical rupture of yeast cells, a flat-roller-drum mill was used. To measure the degree of cell wall disruption, astaxanthin extractability [(extracted carotenoid by ethyl alcohol)/(total carotenoid extracted by dimethyl sulfoxide treatment)] was used. Repeated milling (up to 10 times in the case of plant-scale processing) of the yeast continuously increased the extractability of carotenoids (Fig. 2). By 10 repeated millings, the carotenoid extractability of the yeast subjected to plant-scale spray-drying increased from 0.7 to 76.7% (Fig. 2). As shown in Fig. 1b, plant-scale spray-drying produced various sized clusters in the powder. The smaller cell clusters were flattened by a few repeated millings, but the larger clusters needed repeated milling to crack the cell walls.

To observe the rupture of cell walls, a scanning electron microscopy was used. The shapes of the intact cell clusters

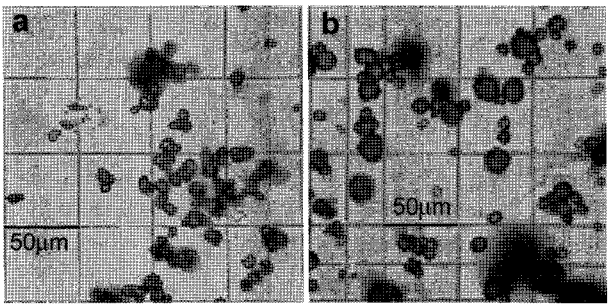

Fig. 1. Sizes of spray-dried cell clusters of *Xanthophyllomyce dendrorhous*. Panels: a, laboratory-scale; b, plant-scale.

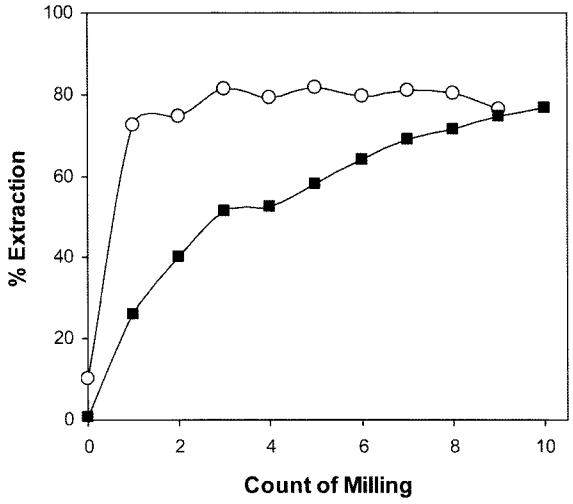

Fig. 2. Effects of milling after spray-drying on ethanol extraction of yeast astaxanthin. Symbols: ■, plant-scale; ○, laboratory-scale. Extraction (%) means the ratio of carotenoid extracted by ethanol to carotenoid extracted after cell wall dissolution by dimethyl sulfoxide.

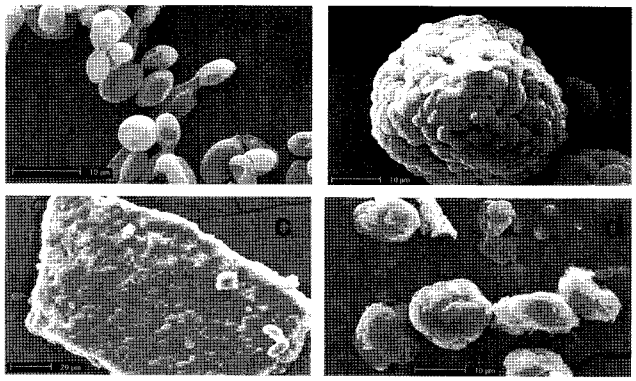

Fig. 3. Shapes of *Xanthophyllomyces dendrorhous* clusters after drying. Panels: a, freeze-dried yeast cells; b, spray-dried yeast cells; c, drum-dried yeast cells; d, spray-dried and milled yeast cells.

prepared by spray-drying were spherical (Fig. 3b). After milling, the spherical cell clusters were flattened and the cell walls cracked by pressing in a flat-roller-drum mill (Fig. 3d). This result indicates that the bigger the cell clusters, the more repetitions are necessary during milling (Fig. 1-3).

Drum-drying the yeast on a hot (150°C) drum surface significantly increased carotenoid extractability (Table 1). Furthermore, the cost of drum-drying was lower than that of spray-drying. The extractability of the yeast prepared by drum-drying prior to milling was higher than yeast that were spray-dried (Table 1). This result demonstrates the increased degradation of the cell wall due to heating. However, drum-drying significantly degraded astaxanthin (residual astaxanthin, 0.82 mg/g yeast) compared to the control (1.60 mg astaxanthin/g yeast) (Table 1). Though the cost of drum-drying was lower and it increased extractability, the increased degradation of astaxanthin made it more difficult to use.

The cell clusters prepared by drum-drying were of an irregular flat-block shape because the cells were dried on a hot drum and scraped off with a knife (Fig. 3c). The shape of cells prepared by freeze-drying or spray-drying were fairly uniform, whereas those prepared by drum-drying were severely distorted (Fig. 3a-c). This result explains why yeast cells prepared by drum-drying had increased carotenoid extractability.

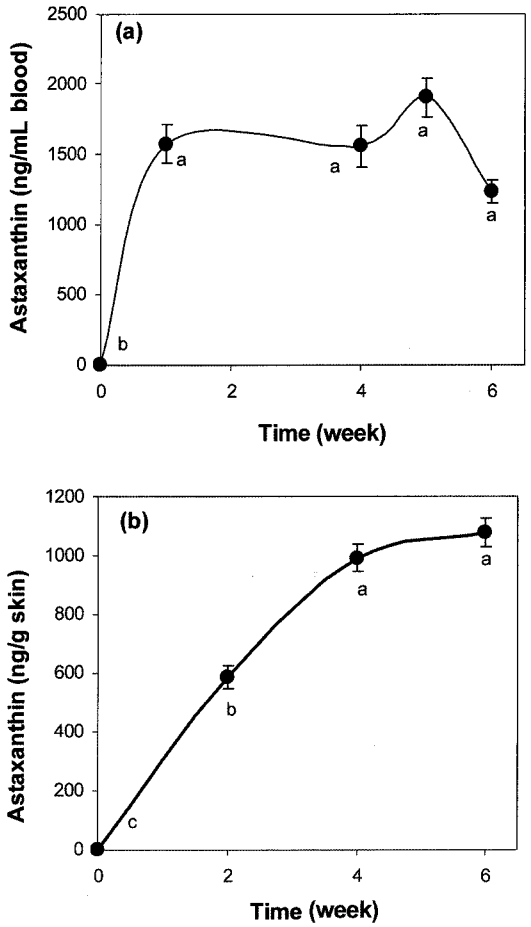

Fig. 4. Astaxanthin in blood and skin after 6-week of feeding to laying hens. Pannels: (a), astaxanthin in blood; (b), astaxanthin in skin. [a,b]Within a variable, bars lacking common letters differ significantly (≤0.05).

Effects of drying and milling on carotenoid availability in animals In preliminary experiments, the astaxanthin content in blood of chickens was increased about 5-fold by milling when the yeast was prepared by spray-drying (unpublished data). To test the availability of astaxanthin from spray-dried and milled yeast in animals, a 6-week feeding trial of chickens was carried out. When astaxanthin was supplied at 40 mg/kg in the feed, it was

Table 1. Effects of various drying methods on astaxanthin extraction and content[1)]

		Astaxanthin equivalent (mg/g yeast)	Astaxanthin (mg/g yeast)
Spray-dry	Ethanol extraction without milling	0.02	0.01
	Ethanol extraction after 10 milling	2.01	1.31
	Dimethyl sulfoxide extraction control	2.32	1.52
Drum-dry	Ethanol extraction without milling	0.31	0.18
	Ethanol extraction after 10 milling	1.26	0.73
	Dimethyl sulfoxide extraction control	1.40	0.82
Control	Dimethyl sulfoxide extraction control	2.41	1.60

[1)]Samples were prepared in triplicate.

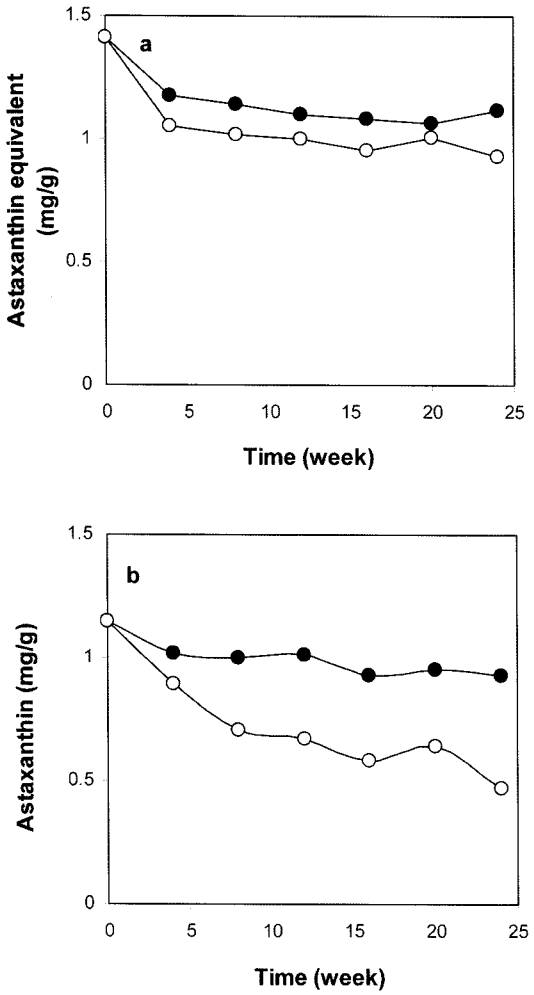

Fig. 5. Astaxanthin during storage. Symbols: ●, 4°C; ○, 30 °C. Pannels: a, Astaxanthin equivalent; b, Astaxanthin

present in chicken blood at a maximum of 1,500 ng/mL after 1-week of feeding (Fig. 4a). The astaxanthin in skin reached a maximum of 1,100 ng/g of skin after 4 weeks (Fig. 4b). Therefore, yeast prepared by spray-drying and milling was successfully used as a pigmentation agent in animals.

Storage of extracted astaxanthin To examine the usefulness of the carotenoid extract as a component of functional food, the extracts were mixed with skim milk powder. The yeast carotenoids were easily extracted in ethanol and then mixed with the skim milk. The storage of the mixture in a refrigerator for 25 weeks caused a 10% loss of carotenoids (Fig. 5). Carotenoids including astaxanthin were significantly degraded during storage at 30°C.

X. dendrorhous (*Phaffia rhodozyma*) is used as a colorant for aquaculture, egg yolks, and crustaceans but its carotenoids can only be absorbed by animals when its cell wall is degraded (3, 7). Chloric acid was effective at degrading the cell wall of this yeast (8). The treatment with chloric acid, neutralization with sodium hydroxide, and spray-drying increased the carotenoid extractability (8). Astaxanthin in the yeast could be made bio-available to laying hens by chloric acid treatment (6). However, treatment with heat and chloric acid degraded a large portion of astaxanthin (45%) (8).

After cell wall degradation by heat and chloric acid, yeast astaxanthin affected the color of muscle as well as the lipid-rich parts (skin and lipid components) (6). When chickens were fed with the yeast (40 mg astaxanthin/kg feed) prepared by drum-drying, astaxanthin was successfully absorbed. When the yeast was prepared by drum-drying, milling did not increase the concentration of astaxanthin in blood and skin (data not shown). The drum-dry method degraded astaxanthin (Table 1), and thus could be used as a simple preparation for an animal feed if a partial loss of astaxanthin is acceptable. If carotenoid extractability was higher than 25% as shown in Table 1, the availability of yeast astaxanthin in animals was good, probably due to the digestive system of the animal.

The absorption of astaxanthin from spray-dried and milled yeast into the blood and skin was superior to that of unprocessed yeast (5-10 fold, unpublished data). For maximal astaxanthin availability and the minimal astaxanthin degradation, milling after spray-drying is preferable. This method can also be used for the extraction of carotenoids in the yeast, since the method is simple and safe.

The cell walls of the yeast were well preserved when they were freeze-dried (see Fig. 3a), however such method was not used in this study due to the expensive cost. Fluid-bed-dry was hard to use for the yeast because of its slimy character requiring supporter starch.

Astaxanthin in blood was directly proportional to the concentration of the yeast in the feed (unpublished data). Supplied astaxanthin reached a maximum in blood after 1 week, and in skin after 4 weeks (Fig. 4). Similarly, Williams *et al.* (13) reported that the absorbed carotenoids by laying hens were transported to the egg-yolk within 48 hr, and carotenoids in egg-yolk reached a maximum concentration after 8-10 days. Furthermore, Hatzipanagiotou and Hartfiel (14) reported that the feeding of carotenoids affected the color of egg-yolks after one day, but feeding for 4-5 day was necessary for stable pigmentation. Ten days after feeding stop, detectable carotenoids were not remained in the bodies of chickens.

With regard to the process of preparing *X. dendrorhous*, we conclude that the drum-dry method could be simply used without milling, in spite of the loss of astaxanthin, and that the spray-dry method was effective with repeated flat-roller milling.

Astaxanthin is sold in liquid capsules (Vitamin World, Inc., New York, NY, USA) and its shelf life is 2-3 years. In this study, astaxanthin was prepared as a powder in capsules, but its shelf life at 30°C was significantly shorter than that of liquid preparations, probably due to contact with oxygen. The astaxanthin in milled yeast packed by vacuum in aluminum packages did not decrease (>1%) over a period of one year (unpublished data). Therefore, astaxanthin produced by the yeast *X. dendrorhous* can be stored in vacuum aluminum packages without extraction.

Acknowledgments

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