Production of Emulsifying and Antioxidative Substances from Defatted Rice Bran by Two-step Subcritical Water Treatment

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Defatted rice bran treated using subcritical water at temperatures between 150 and 180°C for 10 min was further treated under subcritical condition at 250°C for 5 min. The bran extracts obtained from the first-step treatment were evaluated on their emulsifying and emulsion-stabilizing activities by the Pearce and Kinsella method and spectrophotometry at 500 nm. The emulsifying activity of the bran extracts was not dependent upon the first-step treatment temperature. The bran extracts performed equally well in stabilizing emulsions through the test storage period. The antioxidative ability of the bran extracts from each series of the two-step subcritical water treatment was assessed by measuring the induction period and also by monitoring the pressure drop in the closed bottle where oxidation took place. The oxidation was better suppressed in samples added with greater amount of bran extracts. The suppression of coloration of bran extracts by two-step treatment was studied. The bran extracts from the first-step treatment showed high emulsion-stabilizing activity, while the subsequent treatment yielded extracts with a radical scavenging activity comparable to that of the control bran extract obtained from a single-step treatment at 250°C. The two-step subcritical water treatment of defatted rice bran produced higher overall yields compared to the single-step treatment, but showed no noticeable influence upon suppression of coloration in the bran extracts.

Key words: Defatted rice bran, Two-step subcritical water treatment, Coloration, Emulsifying activity, Antioxidative ability

1. Introduction

Consuming rice as the staple, 0.8 million tons of rice bran is produced annually in Japan as a by-product from the rice milling process, half of which is used as the raw material for oil extraction. This brings about roughly 60,000 tons of rice bran oil production a year [1]. Defatted rice bran finds its uses in several applications such as animal feed and fertilizer for mushroom cultivation. Nevertheless, most of it is discarded as an agricultural waste. Defatted rice bran contains various functional substances like ferulic acid, a substance that exhibits antioxidative [2] and UV-absorbing [3] properties, and tocopherol, which is known for its antioxidative property [4]. Defatted rice bran could be considered a promising unused resource. Therefore, continuous efforts have been dedicated by researchers to exploring its effective utilization.

As opposed to the unique supercritical condition of

water above its critical point (374°C, 22.1 MPa), water is present in subcritical condition when being compressed to remain in its liquid state within the temperature range of 100 to 374°C. Subcritical water improves with increasing temperature in its ability to dissolve hydrophobic substances owing to the decrease of dielectric constant. Temperature rise from ambient to 250°C results in a decrease of dielectric constant from 80 to approximately 27, which is almost equal to that of ethanol or acetone at ambient temperature [5,6]. Furthermore, an over 10,000-fold increase in the ion product relative to that of water at ambient temperature makes subcritical water a promising means for hydrolysis and degradation of sugars and proteins [1,7,8]. Our previous work [1] showed that the bran extracts obtained by subcritical water treatment at around 200°C possessed an emulsifying activity, while the ones obtained at around 250°C demonstrated an antioxidative ability.

Manipulating the temperature-dependency of the properties of bran extracts, a two-step subcritical water treatment was attempted with the first-step treatment done at temperatures between 150 and 180°C for 10 min,

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followed by the second-step treatment at 250°C for 5 min. The bran extracts were characterized and further evaluated on their emulsifying and emulsion-stabilizing activities and antioxidative ability. The two-step subcritical water treatment was also studied as a means to suppress coloration of bran extracts during treatment at 250°C, through removal of the possible causes of coloration, sugars and proteins, by the first-step treatment.

2. Materials and Methods

2.1 Materials

Rice bran, defatted by the AOCS official method Ba 3-38 [9], was supplied by Tsuno Food Industries (Wakayama, Japan). L-Ascorbic acid (purity >99.5%) was purchased from Nacalai Tesque (Kyoto). Folin and Ciocalteu's phenol reagent was from ICN Biochemicals (Aurora, OH, USA). Linoleic acid (purity >95%) was obtained from Tokyo Chemical Industry (Tokyo) and trimethylsilyl diazomethane (2 mol/L in hexane) from Sigma-Aldrich Japan (Tokyo). 1,1-Diphenyl-2-picrylhydrazyl (abbreviated hereafter as DPPH), crystallized bovine serum albumin (BSA, 99% purity), sodium dodecyl sulphate (SDS), soybean oil, and all other chemicals of reagent grade were purchased from Wako Pure Chemical Industries (Osaka). Distilled water was used throughout the entire experiment.

2.2 Two-step subcritical water treatment

Apart from the fact the defatted rice bran was treated twice with subcritical water of different temperature ranges, the treatment procedure was basically similar to that reported by Wiboonsirikul *et al.* [10]. The subcritical water treatment was performed in a 117-mL SUS-316 stainless steel vessel (30 mm i.d. × 165.5 mm height) from Taiatsu Techno (Osaka). Briefly, an accurately weighed amount of approximately 7 g of defatted rice bran was added with 70 mL of water into the described vessel. The vessel was tightly closed and heated in a mantle heater to the prescribed temperature ranging from 150 to 180°C for 10 min, exclusive of the heat-up time. After the desired conditions were achieved, the vessel was cooled immediately in an ice bath to 0°C. The content of the vessel was centrifuged at 8500 rpm (RS-18II, Tomy Seiko, Tokyo) for 10 min to obtain the supernatant, which was then clarified by vacuum filtration through an Advantec filter paper (No. 2, 110 mm ϕ , Toyo Roshi, Tokyo). Subsequently, the abovementioned treatment procedures were repeated on the filtration res-

idue at 250°C for 5 min. Vacuum filtration was carried out through an Advantec cellulose acetate membrane filter with a pore size of 0.8 μ m (47 mm ϕ , Toyo Roshi). The liquid bran extracts were kept in at 4°C until further analyses. For evaluation of yield, emulsifying and emulsion-stabilizing activities, and antioxidative ability, the liquid bran extracts were lyophilized to obtain their solid form.

2.3 Evaluation of coloration characteristics of bran extracts

Coloration of the bran extracts was assessed by both visual evaluation and spectrophotometry. A digital camera (PowerShot S2 IS, Canon, Tokyo) was utilized to capture the digital image of the liquid bran extracts for visual comparison of their appearances. The absorbance spectra of the liquid bran extracts versus water as the blank were measured within the wavelength range of 220 to 500 nm using a Shimadzu UV-1200 spectrophotometer (Shimadzu, Kyoto). The liquid bran extracts were diluted 1000 times with water prior to measurement.

2.4 Characterization of bran extracts

2.4.1 Total saccharide content

The total saccharide content in the bran extracts was determined separately for the two steps of treatments. The phenol-sulfuric acid method was employed with some minor modification [11]. One milliliter of the diluted liquid bran extracts or standard glucose solutions was added with 25 μ L of an 80% (w/w) aqueous phenol solution, followed by 2.5 mL of H₂SO₄. After addition, the mixtures were well mixed and held for 10 min under ambient conditions before being cooled in a 25°C water bath for another 10 min. The total saccharide content was determined with the Shimadzu UV-1200 spectrophotometer at 490 nm. All experiments were performed in triplicate.

2.4.2 Total protein content

The total protein content was also assayed separately for the bran extracts obtained from different steps of treatments. The total protein content was evaluated in accordance to the Lowry-Folin assay [12]. The total protein content determined by this method also takes into account the degradation products of protein such as peptides and amino acids. The calibration curve was constructed using standard BSA solutions. Triplicate assays were run for every liquid bran extract.

2.4.3 Yield of extraction

Thirty milliliter of the liquid bran extract was lyophilized. The yield of extraction was computed by calculat-

ing the equivalent weight of solid bran extract for 70 mL of liquid bran extract and dividing the value by 7 g of defatted rice bran. The yield was determined in triplicate and presented in the units of g/g-bran.

2.4.4 Radical scavenging activity

Ten to 160-fold dilution was performed on the liquid bran extracts with 50% (v/v) ethanol solution. Eight tenth of a milliliter of the diluted liquid bran extract or the L-ascorbic acid in 50% (v/v) ethanol solution (VC solution) was added with an aliquot of 0.2 mL of 0.5 mmol/L DPPH in ethanol. The blank comprised 0.2 mL of ethanol and 0.8 mL of 50% (v/v) ethanol solution. The VC solutions were used as the standards for construction of the calibration curve. The mixtures were well mixed and kept in the dark for 20 min at room temperature. The remaining amount of DPPH was quantified with the Shimadzu UV-1200 spectrophotometer at 516 nm. The measurement was performed in triplicate. The radical scavenging activity (%) was calculated from the following equation [13]:

$$\text{Radical scavenging activity (\%)} = (A - B + C) / A \times 100 \quad (1)$$

where *A* represents the initial absorbance of the blank, *B* represents the absorbance of the mixture of the 0.8 mL of diluted liquid bran extract or the VC solution and 0.2 mL of the DPPH solution after 20 min of dark storage, and *C* represents the absorbance of the mixture of 0.8 mL of diluted liquid bran extract or the VC solution and 0.2 mL of ethanol. The radical scavenging activity of the bran extracts was defined as the amounts needed to reduce the initial DPPH concentration by 50% and was expressed in mmol VC equiv./g-bran by referring to the calibration curve of VC solutions.

2.4.5 Emulsifying and emulsion-stabilizing activities

The evaluation of the emulsifying activity of the bran extracts was carried out according to our previously reported method [1], with slight modifications. Three milliliter of 3% (w/v) bran extract solutions were prepared from the solid bran extracts collected from the first-step treatment at 150, 160, 170, and 180°C. Oil-in-water emulsions were prepared by homogenizing 150 mg of soybean oil with 3 mL of the bran extract solutions with a rotor/stator homogenizer (Physcotron NS-50, Nichion Irida Kiki, Tokyo) for 3 min at approximately 25,000 rpm. It has been reported that emulsifying activity of a substance could be evaluated by measuring the absorbance of the emulsion at 500 nm [14,15]. The emulsifying and emulsion-stabilizing activities of the afore-

mentioned bran extracts were evaluated by measuring the absorbance of their corresponding resultant emulsions immediately after preparation and after 20-h storage at 25°C, respectively. Dilution of the emulsions was done with 0.1% (w/v) SDS to produce an absorbance below 0.4. Besides spectrophotometry, the median diameter of the oil droplets in the emulsions was also measured using a SALD-2100 laser diffraction particle-size analyzer (Shimadzu, Kyoto). All experiments were done in triplicate.

2.4.6 Antioxidative ability against oxidation of linoleic acid

Antioxidative ability of the bran extracts was evaluated on the retardation effect upon oxidation of bulk linoleic acid by measuring the induction period based on the accelerated method [16]. The solid bran extract obtained from the second-step treatment with the first-step treatment temperature of 180°C was evaluated. Briefly, 10, 20, and 30 μ L of a 1.0% (w/v) bran extract in 50% (v/v) ethanol solution were added to 100-mL amber vials, each containing 500 mg of a 20% (w/w) linoleic acid in methanol. The mixtures were lyophilized for 6 h, giving the resultant mixtures that contained 100 mg linoleic acid with 0.5, 1.0, and 1.5% (w/w) bran extract, respectively. The amber vials, acting as the reaction vessels, were capped with silicon stoppers and placed in a DNF-400 oven (Yamato Scientific, Tokyo) at 110°C. Clean air was flowed with an AP-032ZN air pump (Iwaki, Tokyo) at the flow rate of 10 L/h first through the vial and then through a glass tube (15 mm ϕ $\times$ 200 mm) that contained 50 mL of water at 25°C. The volatiles resulted from oxidation of linoleic acid in the vial was brought along in the air and trapped in the water in the glass tube. The conductance of the water was measured using an AD-5827 LCR-meter (A&D, Tokyo). The conductivity of the water was recorded every 10 s via the connection of the LCR-meter to a personal computer. All the equipments and glass wares were ensured sufficiently cleaned to avoid any contamination that may catalyze the oxidation of the bulk linoleic acid. The time from the commencement of experiment until the abrupt increase in conductivity was defined as the induction period of oxidation.

As oxidation proceeds in a closed chamber, oxygen is consumed resulting in a drop in pressure. The pressure drop shall be proportional to the degree of progression of oxidation. Antioxidative ability of the bran extracts was also evaluated on the basis of the above-mentioned idea by monitoring the pressure drop in closed 100-mL wide-mouthed bottles where oxidation took place. The solid

bran extracts obtained from the second-step treatment as well as the single-step treatment at 250°C were evaluated. Briefly, three tenth of a milliliter of a 1.0% (w/v) bran extract in 50% (v/v) ethanol solution was added to a 20-mL vial containing 750 mg of a 20% (w/w) linoleic acid in methanol. The mixtures were lyophilized for 6 h, giving the resultant mixtures that respectively contained 150 mg linoleic acid with 2% (w/w) bran extract. Subsequently, the vials were placed into the wide-mouthed bottles, each containing 10 mL of saturated lithium chloride solution. The bottles, to which a micro-manometer (BA Series, AU G1/4 60th -20-20 kPa, Daiichi Keiki Seisakusho, Amagasaki, Hyogo) was mounted on each of them, were then closed and the inner atmosphere of the bottles was ensured to be of atmospheric pressure. The bottles were held in the dark at 50°C in the oven with the inner atmosphere of the bottles controlled at a relative humidity of about 11% by the saturated lithium chloride solution. The pressure drop was recorded from the point when the inner pressure of the bottles stabilized. The control samples were the linoleic acid with no added bran extract. All experiments were done in triplicate.

3. Results and Discussion

3.1 Coloration characteristics of bran extract

The photos of the liquid bran extracts obtained from the first-step and second-step treatment are shown in the upper and lower rows, respectively, in Fig. 1. Generally, the liquid bran extracts from the first-step treatment appeared comparatively lighter in color. The color darkness increased with increasing treatment tem-

perature. At the treatment temperature of 180°C, the color of the liquid bran extract was almost as dark as the one obtained at 250°C and those obtained from second-step treatment. The color darkness is plausibly consequential to the browning reactions, either the Maillard browning or caramelization between the extracted proteins and carbohydrates [17]. Darker color places certain limitations on the application of the bran extracts in food. Hence, the two-step treatment was attempted to curb coloration by first removing carbohydrates, which may be the possible cause of coloration. However, the color of liquid bran extracts from the second-step treatment was seemingly unaffected by the first-step treatment, suggesting the presence of other substances that contributed to the dark color.

Figure 2(a) illustrates the absorption spectra of liquid bran extracts from the first-step treatment at various temperatures. The absorption maxima of the liquid bran extracts prepared from the first-step treatment shifted from 260 nm to 280 nm with increasing first-step treatment temperature, indicating changes in the composition of liquid bran extracts. The absorption maximum at the wavelength around 280 nm is also indicative of the presence of proteins, browning reaction products, and soluble lignin [18–20].

Figure 2(b) shows the absorption spectra of liquid bran extracts from the second-step treatment. The liquid bran extracts obtained from the second-step treatment recorded absorption spectra that resembled each other, except for the one subjected to the first-step treatment at 180°C. The similarity in absorption spectra may imply the presence of similar constituents at different concentrations. In the two-step treatment with the first-step performed at 180°C, the absorption spectra of the liquid bran extracts from the both steps are similar.

Fig. 1 Typical photographs of the filtered liquid bran extracts obtained from subcritical water treatment. Upper row: first-step extraction at (a1), 150°C; (a2), 160°C; (a3), 170°C; (a4), 180°C for 10 min. Lower row: respective second-step extraction of (a1), (a2), (a3), and (a4) at 250°C for 5 min. (c) is the single-step treatment at 250°C for 5 min.

Fig. 2 UV-Vis spectra of the bran extracts obtained from the (a) first-step treatment at temperatures of (1) 150°C, (2) 160°C, (3) 170°C, (4) 180°C for 10, (5) 250°C for 5 min and (b) their respective second-step treatment at 250°C for 5 min.

3.2 Characterization of bran extracts

Figure 3 shows the dependencies of the production yields of saccharide and protein on treatment temperature for both steps of treatment. In the first-step treatment, the yields of saccharide and protein increased with increasing first-step treatment temperature. Both yields were generally higher than their corresponding values at

250°C. However, the decrease in the yield of saccharide was significantly greater due to the degradation of saccharides as opposed to the degradation of proteins to proteinoous substances which are comprehensively detected by the Lowry-Folin method. The yields of saccharide and protein in the second-step treatment were almost constant, which were close to those of the first-step treatment at 250°C. Presumably, the yields of saccharide and protein were limited by degradation of saccharides and the resultant peptides and amino acids at the particular temperature, instead of their respective extractable amounts.

Figure 4 shows the yield of each step of treatment as a function of the first-step treatment temperature. The yield of the first-step treatment increased with increasing first-step treatment temperature, whereas the one of the second step was almost constant. The result could possibly be ascribed to the temperature-dependency of the constituents of the solid bran extracts. The increase in overall yield marked an improvement to the extraction of functional substances from defatted rice bran by the proposed two-step subcritical water treatment.

The bran extracts obtained by subcritical water treatment at around 250°C possess an antioxidative ability. Figure 5 shows the DPPH radical scavenging activity of liquid bran extracts from the second-step treatment. There was almost no difference in the radical scavenging activity among the liquid bran extracts. The strong DPPH radical scavenging activity resulted mainly from the phenolic substances released into the bran extracts

Fig. 3 Total (○, ●) saccharide and total (△, ▲) protein contents of the bran extracts obtained from (open symbols) first-step extraction and (closed symbol) second-step extraction at various first-step extraction temperatures.

Fig. 4 Dependence of yield of the bran extracts obtained from the (○) first-step treatment for 10 min (5 min for that at 250°C) and (●) second-step treatment on the first-step treatment temperature.

Fig. 5 Radical scavenging activity of bran extracts obtained from the second-step treatment as a function of the first-step treatment temperature in comparison to the bran extract obtained from the single-step treatment at 250°C for 5 min.

[10]. At high treatment temperatures like 250°C, the phenolic substances extensively bound to hemicellulose, cellulose, and lignin in the bran cell wall may be released in large amount owing to the hydrolysis of hemicellulose and cellulose. Besides phenolic substances, the products from browning reactions such as protein-carbohydrate conjugates, melanoidins, and heterocyclic compounds may also be partly responsible for the radical scavenging activity [21–23].

In comparison to the bran extract from the first-step treatment at 250°C, there was a decline in radical scavenging activity, though by only about 30%. This finding may partly be attributed to the degradation of phenolic compounds as treatment prolonged [17]. Moreover, part of the phenolic substances may have been extracted during the first-step treatment.

3.3 Emulsifying and emulsion-stabilizing activities

It was previously reported that rice bran extracts prepared using subcritical water treatment at 120 to 180°C for 7.5 min possessed emulsifying and emulsion-stabilizing activities for the preparation of oil-in-water emulsion [16]. The emulsifying activity of the bran extracts obtained from the first-step treatment at 150, 160, 170, and 180°C was evaluated immediately after preparation by the absorbance at 500 nm [14] and also the median diameter of oil droplets of the emulsions (Fig. 6). There is rarely a difference in the absorbance at 500 nm and median diameter of oil droplets among the bran extracts,

implying similar emulsifying ability of the bran extracts.

The emulsion-stabilizing activity of the bran extracts was estimated by the same methods after a storage period of 20 h at room temperature as presented in Fig. 7. Both the absorbance and median diameter stayed consistent through the 20-h storage period, substantiating the emulsion-stabilizing activity of all the tested bran extracts.

Fig. 6 Emulsifying activity of bran extracts obtained from the first-step treatment at various temperatures assessed by (Δ) spectrophotometry at 500 nm and ($\circ$) oil droplet size analysis.

Fig. 7 Emulsion-stabilizing activity of bran extracts obtained from the first-step treatment at various temperatures assessed by (Δ) spectrophotometry at 500 nm and ($\circ$) oil droplet size analysis of the emulsions immediately after preparation and after a 20-h storage.

3.4 Antioxidative ability against oxidation of linoleic acid

Figure 8 demonstrates the typical changes in conductivity for bulk linoleic acid added with the bran extract obtained from the second-step treatment with the first-step treatment temperature of 180°C at the concentrations of 0.5, 1.0, and 1.5% on weight basis. The inflection points in the conductivity curves represent the induction periods of oxidation in the respective systems. Longer induction period reflects better stabilization of the acid against oxidation. All the bulk linoleic acid added with the bran extract showed improved stability against oxidation (induction period: control (0% (w/w)), 36 min; 0.5% (w/w), 63 min; 1.0% (w/w), 138 min; 1.5% (w/w), 144 min). The induction period prolonged as the added amount of bran extract increased, but not in a proportional manner. At the addition of 1.5% (w/w) of bran extract, only a slight improvement in terms of induction period was effected in comparison to the sample added with 1.0% (w/w) of bran extract. However, the increase in conductivity after the induction period was found to occur at a slower rate.

The induction period of the bran extracts obtained from the two-step treatment with various first-step treatment temperatures was plotted as a function of the amount of bran extract added to the bulk linoleic acid (Fig. 9). Regardless of the first-step treatment temperature, all bran extracts showed similar improvement in stabilizing bulk linoleic acid against oxidation. The stabi-

Fig. 8 Typical changes in the conductivity for bulk linoleic acid containing (○) 0% (w/w), (△) 0.5% (w/w), (□) 1.0% (w/w), and (▽) 1.5% (w/w) of bran extract obtained from the second-step treatment with the first-step treatment temperature of 180°C.

lization effect is dependent upon its concentration in the system rather than the first-step treatment temperature.

The antioxidative ability of the bran extracts obtained from the second-step treatment with different first-step treatment temperatures was also estimated by monitoring the pressure drop in the closed wide-mouthed bottles throughout the oxidation reaction (Fig. 10). A steep

Fig. 9 Induction period of oxidation reaction of bulk linoleic acid added with bran extracts obtained from the second-step treatment with the first-step treatment temperatures of (□) 150°C, (▽) 160°C, (◇) 170°C, (△) 180°C, and (○) the control sample from the first-step treatment at 250°C for 5 min.

Fig. 10 Pressure drop in the closed wide-mouthed bottles resulted from consumption of oxygen during oxidation of bulk linoleic acid containing 2% (w/w) of bran extracts obtained from the second-step treatment with the first-step treatment temperatures of (□) 150°C, (◇) 170°C, (△) 180°C, and (○) the positive control sample from the first-step treatment at 250°C for 5 min. (●) is the negative control sample without addition of bran extract.

decrease in pressure was recorded for the negative control sample. The bran extract from the single-step treatment at 250°C for 5 min was used as the positive control. The bran extracts obtained from the two-step treatment were equally as effective as the positive control in retarding oxidation of bulk linoleic acid. The significantly different curve patterns of samples added with bran extracts from that of the negative control further verified the antioxidative ability of the bran extracts.

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◆◆◆ 和文要約 ◆◆◆

亜臨界水を用いた脱脂米糠の二段階処理による 乳化性および抗酸化性物質の生産

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常圧での沸点である 100℃から臨界温度である 374℃の範囲で加圧することにより液体状態を保った水を亜臨界水または加圧熱水という。亜臨界水とよぶ温度範囲については、明確な定義がないように思われるが、ここでは上述の範囲とする。常温常圧の水に比べて、亜臨界水は次の 2 つの特徴を有する。1 つは温度の上昇とともに比誘電率が著しく低下し、200℃以上ではアセトンなどの水溶性有機溶媒のそれとほぼ同程度の値になる。この特性から、亜臨界水には疎水性物質が比較的高濃度で溶解する。もう 1 つの特徴は、イオン積が大きいことであり、200℃～300℃では $10^{-11}(\text{mol/kg})^2$ のオーダーとなる。すなわち、亜臨界水は水素イオンおよび水酸化物イオンの濃度が高く、高温であることが相俟って、水自体が酸または塩基触媒として作用し、加水分解のみならず、分解や異性化などの反応を触媒する。このような特性に基づき、亜臨界水を用いて食品残渣や農産廃棄物から有用物質を抽出しようとする研究が数多くなされている。なお、亜臨界水を用いた処理では、(加水) 分解と抽出が同時に起こると考えられるが、操作の目的より抽出と表記されることが多い。

著者らは、利用価値の低い農産(廃棄)物である脱脂米糠や小麦フスマの亜臨界水処理による有用物質の抽出と抽出物の利用に関する検討を行っている。脱脂米糠を 150℃～180℃程度の比較的低温で処理すると、糖質の含有率が高く、乳化性および乳化安定性を有する物質が得られる。一方、250℃前後の高温で処理すると、Lowery-Folin 法で呈色するタンパク質性の物質(必ずしも高分子とは限らず、ペプチドやアミノ酸も含まれる可能性がある)の割合が高く、抗酸化性をもつ物

質が得られる。これらの処理における抽出物の収率は、温度にも依存するが、概ね 20%から 30%である。しかし、抗酸化性をもつ物質を得るために 250℃前後の高温で処理すると、Maillard 反応などに起因すると推測されるが、抽出液は著しく褐色を呈しており、用途の限定が危惧される。そこで、脱脂米糠を 150℃～180℃程度の比較的低温で処理して、糖質が多く乳化性などをもつ抽出液を得たのち、その残渣に加水してさらに 250℃前後で処理すれば抗酸化性をもつ抽出液が得られるとともに、二段階目の処理では脱脂米糠中の糖質の含有率が低下しているため、着色が抑制できないかと着想した。

150℃～180℃で処理することにより、既往の知見通りに、乳化性と乳化安定性をもつ物質が得られた。その残渣に一段階目の処理で回収した抽出液と同量の水を加えて、さらに 250℃で処理したところ、抗酸化性をもつ抽出物が得られた。しかし、二段階処理により着色を抑えるという所期の目的は実現しなかった。これは一段階目の処理で抽出されなかった残渣にまだ多くの糖質が残存しており、それらが二段階目の処理で切り出されて低分子化して生成した単糖などが分解して着色物質を生成するとともに、タンパク質性の物質などと Maillard 反応などを起したためと推測される。一方、脱脂米糠を二段階で処理することにより、一段階目と二段階目の抽出物の和で計算した総括的な収率は 50%前後と著しく向上した。二段階処理は操作が煩雑であるという問題はあるが、廃棄物量を大幅に低減でき、かつ乳化性と抗酸化性という異なる機能をもつ物質が得られるという点では利点があると考えられる。

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