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Free fatty acids in rice bran during its storage after a treatment by twin-screw extrusion to prevent possible rapid hydrolytic rancidity of lipids

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Abstract
This research studied the prevention of hydrolytic rancidity during the storage of rice bran stabilized by an extrusion process. A double screw extruder was used, set at 130° C in the barrel and a rotational speed of the screws programmed at 200 RPM. The moisture content of the bran was adjusted at 20%. The treated bran was stored, using two packing methods (with and without vacuum), at two ambient conditions (18° C – 70 % RH and 30° C - 78% RH) for a period of 8 weeks. The content of free fatty acids (FFA) in the rice bran was observed during the storage time period. The extruded treated rice bran stored at 18° C - 70% RH resulted in lower values of FFA in comparison with the values in the bran kept at 30° C - 78% RH. Vacuum packaging showed a significant disadvantage as compared to packaging without vacuum.

Keywords: rice bran; extrusion; free fatty acids; hydrolytic rancidity; packaging; storage.

Ácidos grasos libres del salvado de arroz durante el almacenamiento después de un tratamiento por extrusión para prevenir la rápida rancidez hidrolítica de los lípidos

Resumen
Esta investigación estudió la prevención de rancidez hidrolítica durante el almacenamiento del salvado de arroz extruido. Se usó un extrusor de doble tornillo, ajustado a 130° C en el barril y una velocidad de rotación de los tornillos de 200 RPM. El contenido de humedad del salvado se ajustó al 20%. El salvado tratado se almacenó utilizando dos métodos de envasado (con y sin vacío), en dos condiciones ambientales (18° C - 70% HR y 30° C - 78% HR) durante un período de 8 semanas. El contenido de ácidos grasos libres (AGL) en el salvado de arroz se observó durante el almacenamiento. El salvado tratado extruido almacenado a 18° C - 70% de HR presentó valores más bajos de AGL en comparación con los valores del salvado a 30° C - 78% de HR. El envasado al vacío mostró una desventaja significativa en comparación con el envasado sin vacío.

Palabras clave: salvado de arroz; extrusión; ácidos grasos libres; rancidez hidrolítica; empaque; almacenamiento.

1. Introduction

Rice bran (RB) is a by-product of the rice milling process, it amounts to nearly 7-8% of the rice grain [1], and is a source of protein, fiber and lipids, basically unsaturated fatty acids [2]. After its production in the mill it undergoes rapid enzymatic hydrolysis caused by lipases activity on the bran lipids released from the cells in the milling process, which results in the decomposition of lipids into free fatty acids (FFA) [3]. Therefore, because of the biochemical instability of rice bran, occurring immediately after the polishing of rice grain, it has a short shelf life due to the decomposition of its lipids which renders it unsuitable for human consumption. However, if the bran is subject to a thermal treatment of high temperature and short time immediately after polishing, the lipase activity is reduced, thus producing a bran with longer shelf life which is suitable for human consumption. Thermal

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treatments to bran rice for stabilization has been applied throw microwaving, extruding, steaming and parboiling [4-11]. Chemical treatments have been also used to prevent rancidity of rice bran [12,13]. This study was conducted to determine the effect on the content of FFA resulting from any residual enzyme activity in fresh rice bran after being thermally treated by a double screw extruder and stored for a period of time under certain specific conditions. The treated rice bran was stored for a period of two months, packed following two different packaging methods (with and without vacuum) and two ambient conditions (temperature and relative humidity). Rice bran samples preserved by the procedure described above were used in other project, oriented to the development of a ready-to-eat cereal processed by the extrusion and enriched with fibers from rice and maize brans.

2. Materials and method

2.1. Raw material

Fresh RB was obtained from a rice milling factory located in the town of Jamundí, Valle del Cauca, Colombia. The RB used was obtained directly on the same day of processing in the mill, which corresponds to the material removed in the polishing step of the rice grain processing. The collected samples were immediately transported to the laboratory at the Universidad del Valle and processed within a maximum period of three hours after removal in from the milling process**.**

2.2. Stabilization by extrusion

Samples of 1 kg of raw bran were heat treated by a twin screw extruder (DS 32-II Jinan Saixin Machinery Co., China) at a rotation speed of the screws of 200 RPM and a barrel temperature of 130° C. The feed rate of bran into the extruder was kept constant at 150 g/min, according to preliminary tests. The moisture content of the bran was adjusted to 20.0 % (w.b.) following a protocol specified in preliminary studies [14].

The extruded bran was immediately cooled to room temperature (26-30° C). Subsequently, the tempered extruded material was left in a oven at 50° C for 24 hours to adjust the moisture to about 5-7% (w.b.).

Figure 1. Twin-screw Extruder Equipment DS 32II
Source: The authors

Table 1**.**
Fatty acid profile for stabilized rice bran**.**

Parameter	Content (%)
Lauric	0,02
Myristic	0,31
Pentadecanoic	0,02
Palmitic	17,32
Palmitoleic	0,17
Heptadecanoic	0,05
Heptadecenoic	0,01
Stearic	1,88
Oleic	43,43
Linoleic	32,82
Linolenic	1,43
Arachidic	0,78
Gadoleic	0,52
Behenic	0,25
Lignoceric	0,02

Source: The authors.

2.3. Composition of fatty acids of stabilized rice bran

The fatty acid content of the rice bran extruded under the said operational conditions was determined (Table 1), prior to the storage and packing tests. The analysis was carried out by a local private laboratory, where standard AOAC methods were used [15]

2.4. Storage of treated rice bran

The RB were packaged (laminated bags metalized) and properly labeled, according to the type of bran (extruded and non–extruded, used as a control treatment), method of packaging, time length and environmental conditions of the storage. Samples were separated into two parts. Half of them was stored in an environmental chamber at 18° C – 70 % RH and the other half was stored at 30° C – 78 % RH, both for 8 weeks.

2.5. Determination of FFA

The enzymatic activity of lipases was determined by measuring the FFA content in the lipids present in the sample. For its determination lipids were extracted using the soxhlet method. 15 g samples of bran were processed for six hours with 200 mL of petroleum ether. To remove the solvent residue in the lipid extracted the ball was placed in an oven at 65° C for 24 hours. The content of free fatty acids was obtained following the AOAC official method [16] by dissolving the lipid extracted into a mixture of 20 mL of ethanol-diethyl ether and, three drops of phenolphthalein. After neutralization with NaOH a slight pink color was reached. The titration for each sample was performed in duplicate.

The FFA content was determined using the eq. (1) and estimated in oleic acid (predominant acid in the lipids of the RB).

$$FFA\ (\%) = \frac{(mL\ sample - mL\ blank) * 28{,}2 * NaOH\ (N)}{Lipid\ weight\ (g)} \quad (1)$$

The samples were analyzed for FFA content (%) over a storaging time of 0 to 8 weeks, at 2 week intervals.

2.6. Experimental design

A factorial design 2x2x2 was used. The independent variables were the type of rice bran (untreated and treated by extrusion), packaging method (with or without vacuum) and two environmental conditions controlled by the temperature and the relative humidity of air. Sampling was done at 0, 2, 4, 6 and 8 week intervals. The response variable was the predominant FFA content of the sample (% Oleic Acid), the measurement was made in triplicate. Minitab version 16, with a confidence level of 95%, was used for the statistical data analysis.

An analysis of variance was carried out, followed by the Tukey test to find out whether there were any similarities between the levels of a single factor (type of rice bran, packaging method and ambient condition) with a confidence in the result of 95 %.

3. Results and discussion

The effects of extrusion on bran stability as measured by the FFA content of stored samples are shown in Figs. 2 and 3.

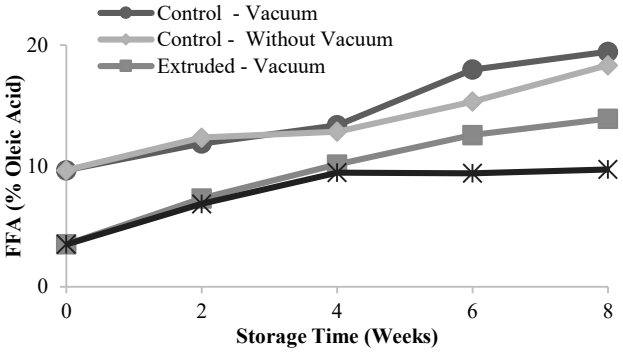

Figure 2. Content of free fatty acids in raw rice bran (control) and extruded rice bran, packaged with and without vacuum, and stored at 30° C – 78 % RH. Source: The authors.

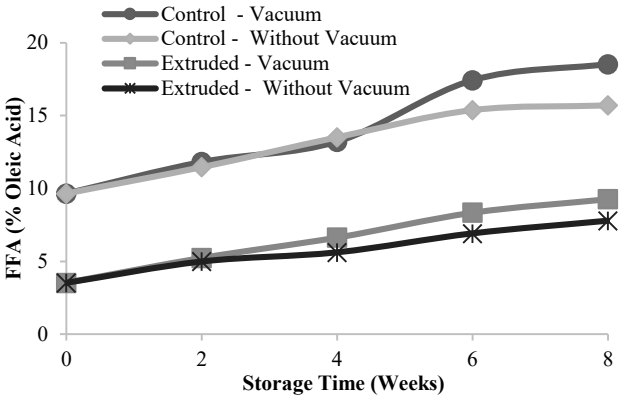

Figure 3. Content of free fatty acids in raw rice bran (control) and packed in extruded rice bran, packaged with and without vacuum, and stored at 18° C – 70 % RH. Source: The authors.

It was found that there was a difference between the two levels of each factor during storage of the samples of bran in function to the contents of FFA. The statistical analysis indicated that the packaging method showed a significant difference ($p < 0.05$) in the content of FFA in the stored bran samples, in both raw and extruded ones, and at both ambient storing conditions. The untreated rice bran showed a significantly higher increase in FFA content compared to the heat-treated rice bran extrusion, regardless of packing methods at both ambient storage conditions. Throughout the experimental period, the lower values of FFA content were observed for the extruded samples stored at 18° C - 70% RH and packaged without vacuum.

Figs. 2 and 3 it is show a constant increase in FFA levels for both packaging methods during the four-week storage period; week 6 and week 8, an accelerating rate of increase of the FFA content was observed in the bran extruded and packed under vacuum and a significantly higher levels of FFA were presented in the extruded sample, packaged without vacuum when stored at 30° C - 78% RH (Fig. 2). The FFA content in bran extruded samples kept in a vacuum packaging was higher than those of the samples stored in laminated bags metalized without vacuum and kept at 18° C – 70 % RH for 8 weeks (Fig. 3). Changes in FFA contents in samples were significant between the packing methods at both ambient conditions. Additionally, the FFA content of samples kept in the vacuum packaging was consistently higher than that in the samples without vacuum packaging over an 8-week storage period at both ambient conditions 18° C – 70 % RH and 30° C – 78 % HR (Fig. 2 and 3).

The increased content of FFA of rice bran without heat treatment by extrusion during the storage test in this study is similar to the results reported in other studies [3,5,17-19]. It is a typical trend the rapid development of FFA caused by hydrolytic rancidity in raw rice bran makes this material unsuitable in food formulations for human consumption [18,20] also observed that in rice bran without being processed by the extruder, there was a decrease in the enzymatic activity of lipases as the storage temperatures were lower.

The FFA content in samples stored in both packaging methods was lower when the samples were kept at 18° C – 70 % (Fig. 3). While in the sample stored at 30° C - 78% the level of FFA increased in a non-vacuum packaging, the increase of FFA was higher in samples kept under the vacuum-packing (Fig. 2). Sharp and Timme [21] noted the same pattern in brown rice stored in bags without vacuum versus those in vacuum packaging. Ramezanzadeh et al. [5] reported a similar behavior in the raw rice bran treated by microwave heating and stored in bags with hermetic sealing and in bags with vacuum sealing and temperatures of 4-5° C and 25° C for 16 weeks. This could be due to the removal of air and oxygen in vacuum packaging, thus observing that the bran samples untreated by extrusion had a possible activation of anaerobic microorganisms that caused increased lipase enzyme activity in the samples of rice bran.

Chahinian et al. [22] in studies on lipase production by microbial metabolism noticed that in the type I lipase, which specifically acts on triacylglycerols, the microbial culture grew without aeration. Lipase type II, which is only activated

partially on acylglycerols, had an optimum growth in microbial culture with aeration, thus suggesting that in the storage process of rice bran, lipases with greater proportion of type I are activated. Additionally, Randall et al. [20] performed a count of anaerobic microorganisms in raw rice bran and extruded one, with results of 82 x 10^3 and 9 x 10^2 bacteria/g, respectively, which certifies high levels of microorganisms growing in a culture medium without the presence of air in the raw bran.

After 8 weeks of storage, in extruded rice bran, a FFA content level of 13.9 and 9.7% in samples packaged with and without vacuum was obtained, respectively, when stored at 30° C - 78 % (Fig. 2) and a FFA level of 9.3 and 7.8% for samples packaged with and without vacuum, respectively, and stored at lower temperature and relative humidity storage (18° C - 70%) (Fig. 3). Bran oil with an excess of 10% of FFA is considered unfit for human consumption [18]. The rate of formation of FFA in the bran or brown rice flour is high. Approximately 30% of lipids may be hydrolyzed to FFA within a week under conditions of high humidity and temperature [3]. From the above it can be said that at the end of a period of two months, the bran extruded and stored at the conditions of 30 ° C - 78% does not meet the requirements for human consumption. On the other hand, it comes from storage with acceptable levels of FFA at an environment of lower temperature and relative humidity. In this case, rice bran is suitable for later use in the manufacture of products for human consumption, for example mixtures of ready-to-eat extruded cereals.

4. Conclusions

FFA values obtained in this study showed that a heat treatment by a twin screw extrusion process may be used as a method for inactivation of lipases to extend the shelf life of the rice bran. Storage at 18° C – 70 % RH resulted in lower values of FFA for an 8-week storage as compared with values of FFA at 30° C – 78 % RH. Vacuum packaging of rice bran showed significant disadvantage as compared with packaging it without vacuum. Therefore, based on the tests used in this study, it can be concluded that the recommended storage conditions to prevent hydrolytic rancidity in the stabilized rice bran by extrusion is the use of laminated bags metalized, packaged without vacuum and at temperature - humidity storage of 18° C – 70 %, for up to 8 weeks of storage.

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