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**EVALUATING CHANGES OF USING RICE BRAN PROTEIN
CONCENTRATE ON DIFFERENT SAUSAGE TREATMENT
FORMULATIONS IN MICROBIAL AND
PHYSICOCHEMICAL PROCESS**

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ABSTRACT

With regard to nutritious value and operational properties of rice bran protein concentrate, extracted by alkaline method, the present study has applied rice bran protein concentrate in formulation of meat productions (conventional sausage). Control sample didn't contain the mentioned protein concentrate; although, the protein was applied one time as an alternative for gluten and one time as an alternative for soya isolate in formulation of produced sausages for 1 %. The sausage was produced in a manufacturing company and the samples were heated in 80°C for 2h. Afterwards, some tests were performed on the samples such as chemical test, physical test, microbial test, and sensory evaluation. The main objective of the present study has been to evaluate changes of different formulations of sausage treatments in microbial and physicochemical process. The main results of the study are as follows: treatments 2 and 3, containing 50gr/5kg rice bran protein concentrate, can be replaced instead of gluten and soya isolate respectively. They are also significantly different from control sausage and both of them can be applied in meat industries.

Key Words: Rice Bran Protein, Sausage Treatments, Microbial Process, Changes of Treatment Formulation

INTRODUCTION

There are no literature in regard with extraction of rice bran and its application in sausage in Iran; although, some studies have been conducted in regard with extraction of rice bran oil. Through having an overview on existed literature, one can found that extraction of rice bran through using protease, containing Exoproteases and Endoproteases, can enhance extraction process from 60% to 93% (Hamada 1999). Due to previous studies, different healthy compounds have been applied, which their application in meat products is considerable. For example, in many studies different alternatives for fat, based on carbohydrate materials, have been applied.

This would lead to reduction in fat content in production without any effect on its technologic and sensory properties. In a study, for reduction of meat fat in emulsion systems from 30% to 20%, mixture of grape's seed oil and 2% of rice bran fiber has been applied. Finally, chemical compounds, cooking properties and physicochemical properties of viscosity fiber have been evaluated. For low-fat meat pastes, containing grape seed oil and rice bran fiber, amounts of ash, pH value in cooked and uncooked samples, viscosity, biting ability and solvability of sarcoplasmic proteins were higher than control sample. Therefore, it should be mentioned that fat reduction from 30% to 20% with applied alternatives would affect efficiently quality of meat pastes (Choi *et al.*, 2010). In a study, effects of rice bran fiber on induced gel by heating solvable proteins in salt of pork have been evaluated. 0.1%, 0.5%, 1% and 2% of rice bran fiber have been added at the same time to solvable meat protein in salt. Moisture content was same in all samples. Solvability of protein L* and pH value were raised through enhancement of rice bran fiber value; although the highest moisture content of myofibril protein in all treatments was depended on adding 1% of rice bran fiber. Although brightness and redness value of texture were reduced through increase in rice bran fiber value. In this regard, electrophoresis gel caused no change in protein, regardless rice bran fiber

Research Article

(Choi *et al.*, 2011). Daneshvar and Azizi (2001), has evaluated effects of wheat powder and fresh vegetables on metabolic and physicochemical quality of waste materials of colon. Obtained results indicated that digestive system would be improved through using wheat bran. Due to the study, using wheat bran (daily 2 teaspoons for 3 months) has been suggested for adults in different ages (Daneshvar and Azizi, 2001).

Main Body

According to national standard No.2303 of Iran, sausage and frankfurter are sustainable combinations of meat fat and water, which would be filled by some other materials in adequate artificial or natural covers. The products would be prepared for consumption after adequate heating process and other required processes. Based on consumptive meat value in this formulation, the products would be classified in four groups as follows: (Iran Standard Organization 2303)

- 1- At least 40-50% meat
- 2- More than 51-60% meat
- 3- More than 61-80% meat
- 4- More than 81-90% meat

Manufacturing sausage and frankfurter needs adequate raw materials, so that desirable quality and safety of product can be provided and it can be cost effective. Sausage and frankfurter can be produced using various kinds of humid, semi-dry and dry raw materials. High amount of raw materials can be combined with each other in different ratios (Mohammadi and Hosseini, 2009). The main negative effect of using meat in meat productions is its high fat value, which would lead to development of some illnesses. For example, animal fat is one of the main reasons for cardiovascular diseases.

Overweight, obesity and other diseases related to food diet with high fat such as different kinds of cancers (colon cancer, prostate cancer, breast cancer, etc), gallbladder diseases, hypertension, insulin resistance, can have the most negative effects on meat product industries and can also lead to reducing acceptance of such products by consumers and their tendency for using low-fat foods (Calorie Control Council, 2007; Garcia and Totsaus, 2007; Ker *et al.*, 2005; Vural *et al.*, 2004; Tokusoglu and Mattes, 1998; Troutt *et al.*, 1992). Need to using low fat foods is not also a public belief, but also many relevant organizations have also advised low-fat foods. Some organizations include American Heart Association, American Cancer Society, American Dietetic Association, World Health Organization, National Academy of Sciences, United States Department of Agriculture, and National Cholesterol Education Program. Accordingly, received cholesterol value would be 300mg daily (Cengiz and Gokoglo, 2005; Calorie Control Council, 2007; Serdaroglu and Ozsumer, 2003). Manufacturing products with low fat value and high protein value, as new and various formulations are also significant economically.

MATERIALS AND METHODS

Methodology

Test for Rice Bran Protein

One experiment was done on rice bran protein concentrate as follow:

Determining Nitrogen Solvability Index

This would be measured according to Bera and Mukherjee approach. First, 20mg of rice bran protein concentrate sample was mixed in 2ml deionized water (DI) respectively in different densities of 0.1, 0.5, or 1m NaCl solvent. Next, pH was regulated on 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0 using HCl or NaOH (1.0, 0.1, or 0.01N).

Then the mixture was mixed in ambient temperature of 25°C with velocity of 250 rpm for 30min. finally, the mixture was centrifuged with velocity of 4000g for 30min and then Nitrogen content of the supernatant was measured by Kjeldahl method. Then index of nitrogen solvability was calculated as follows:

Nitrogen solvability index % = nitrogen of supernatant (mg) / total nitrogen in 100mg of sample × 100.

Research Article

Final Tests for Sample Sausages

Chemical Tests

Before conducting tests, preparation and homogenization of samples based on AOAC (983.18) standard was done. For chemical analysis and measuring fat rate of sample, extraction of fat was conducted by petroleum ether solvent through standard method of AOAC No.991.30 Protein rate of homogenized sample based on standard AOAC No.981.10 based on Kaji-dhal method. Moisture of homogenized sample was measured in oven with 125°C based on standard AOAC No.950.46. Ash rate of sample was measured based on AOAC method No.920.153. Ash rate was measured through mentioned method using electrical oven under temperature of 550 °C. 10gr of homogenized sample with distilled water was achieved to volume of 100ml; pH rate of the mixture was measured through using digital pH meter machine Metrohm Hershia E532, equipped by Metrohm 6.0202.100 electrode (Khalil, 2000).

Color Measuring Test

Evaluation of color variations in productions and assessing effects of rice bran protein have been done by Hunter Lab machine on some pieces of products and three factors L*, b*, and a* have been measured during the test. Color measuring test has been iterated 3 times for each formulation. Therefore, 9 tests have been conducted on each formulation and totally 27 tests have been done (Serdaroglu and Ozsumer, 2003; Grigelmo-Miguel *et al.*, 1999).

Texture Measuring Test

In evaluation process of samples Warner-Bratzler has been applied. The test has been conducted Texture Analyzer machine in ambient temperature on samples with thickness of 4cm and diameter of 2.6cm. Two factors of shear force and required energy for shear have been measured respectively by peak point of the curve and lower surface of the curve calculations. Texture measuring test has been iterated 4times for each formulation and totally the test was iterated for produced samples in 12 iterations (Serdaroglu and Ozsumer, 2003).

Sensory Test

Sensory evaluation of samples has been conducted by 20 specialist and professional analyzers through using Hedonic Test. Samples from 3 formulations, which have been produced in 2 iterations, have been presented in form of some shears in some white plates. In this regard, evaluated properties include taste, smell, color and texture. Scores have been considered from 1 to 6 (worth to excellent) (Sharma, 2000; Garcia *et al.*, 2002; Serdaroglu and Ozsumer, 2003; Caceres *et al.*, 2006).

Microbial Test

Microbial test of products has been conducted according to standard 2303 and Iranian Industrial Researches Organization. In this stage, total number of some bacteria has been considered for all samples. The bacteria included mold, yeast, Staphylococcus aureus, Salmonella, Clostridium Prem Franz, Escherichia coli, and coliform.

Properties of Raw Materials in Sausage Production Process

-Meat

Applied meat has been filet of shoulder meat of young male calf (beef).

Meat preparation stage: prepared meat of fresh carcass, which a part of its fat was separated by hand, was grinded by meat grinder below 0°C and then was grinded by meat grinder above 0°C with 5mm thickness, made in U.S, so that to obtain a uniform mixture.

-Oil

Liquid edible vegetable oil of soya was provided from Isfahan industry of vegetable oil.

-Water

A part of applied water was related to moisture of meat and other part was in form of ice, added to formulation.

-Soya isolate

Packed soya isolate in 20kg packages from Wachsen Industry Co was provided.

Research Article

-Sodium polyphosphate

Sodium polyphosphate was provided from Budenheim Co Germany in 25kg packages.

-Ascorbic acid

Ascorbic acid was provided from Hebi Welcome Pharmaceuticed Co China in 25kg packages.

-Sodium nitrate

Sodium nitrate was provided from Ludwighshafen Co Germany in 25kg packages.

-Additive mixture

Adding additives would lead to provide a good taste and smell for sausages, which include red pepper, thyme, mustard, and Indian nutmeg provided from India, Malaysia and Iran in powder form.

-Salt

Applied salt was provided from Iranian Salt Refinery Company under the term of “refined and washed salt”, in 25kg packages.

-Gluten

Gluten was provided from Parsian Gluten Starch Co Semnan, in kind of active gluten in 25kg packages.

-Starch

High quality starch was provided from Faradaneh Co Shiraz in 25kg packages.

-Wheat flour

Wheat flour was provided from Grain Organization of Iran in 25kg packages.

-Bell pepper and garlic powder

Bell pepper and garlic powder were provided from Morvaride No Co following order of Negine Bartar Yekta Co.

-Stabilizer

Stabilizer was provided from Karma Farayand Co with cooperation of Media Models Co Canada in 20kg packages.

-Rice bran protein concentrate

Applied protein was provided from Shiroodi rice bran through alkaline extraction method.

Properties of Chemical Materials

Applied chemical materials were provided from Merck Co Germany.

Table 1: Properties of applied chemical materials in the study

Row	Chemical material	Company
1	Concentrated sulfuric acid	Merck
2	Boric acid	Merck
3	Sulfuric acid tetrazole 0.1 normality	Merck
4	Sodium hydroxide	Merck
5	Buffer solvent pH=4.00±0.02	Merck
6	Buffer solvent pH=7.00±0.02	Merck
7	N-hexane	Merck
8	Protein reagent	Merck
9	Filter paper	Whatman
10	Brad-parker	Merck
11	Egg emulsion and potassium Tellurite	Merck
12	Lauryl sulfate broth	Merck
13	Peptone water	Merck
14	Rappaport broth	Merck
15	Salmonella-Shigella agar	Merck
16	SPS agar	Merck
17	Plate count agar	Merck

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Formulation of Sample Sausages

To produce the product during primary tests, formulation of German sausage was selected based on table 2.

Table 2: Formulation of German sausage (conventional sausage)

Row	Materials	Values (%)
1	Meat	40
2	Oil	15
3	Starch	10
4	Gluten	1
5	Soya isolate	1
6	Additive mixture	0.75
7	Sodium polyphosphate	0.4
8	Water	23.293
9	Sodium nitrate	0.012
10	Stabilizer	1
11	Bell pepper	1
12	Garlic	1
13	Flour	4
14	Salt	1.5
15	Ascorbic acid	0.045
Total		100

Two formulations have been considered for production of enriched sausage with rice bran protein. The 2 formulations, along with formulation of control sausage have been iterated 2 times in batches of 5kg. In the mentioned two formulations, in addition to fix rate of applied meat, rice bran protein concentrate has been applied one time instead of gluten and another time instead of soya isolate.

- 1) Formulation No1 (control sample formulation): containing 40% meat, 1% soya isolate and 1% gluten as formulation of control sausage
- 2) Formulation No2: containing 40% meat and 1% rice bran protein as enriched sausage instead of gluten
- 3) Formulation No3: containing 40% meat and 1% rice bran protein as enriched sausage instead of soya isolate

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Table 3: Compounds of sample sausages' formulation

Row	Compounds (gr)	Formula	1 (control sample)	2 (enriched sausage of 1% instead of gluten)	3 (enriched sausage of 1% instead of soya isolate)
1	Meat		2000	2000	2000
2	Liquid oil		750	750	750
3	Starch		500	500	500
4	Gluten		50	-	50
5	Soya isolate		50	50	-
6	Additive mixture		37.5	37.5	37.5
7	Flour		200	200	200
8	Sodium polyphosphate		20	20	20
9	Sodium nitrate		0.6	0.6	0.6
10	Water		1164.65	1164.65	1164.65
11	Ascorbic acid		2.25	2.25	2.25
12	Salt		75	75	75
13	Stabilizer		50	50	50
14	Bell pepper		50	50	50
15	Garlic		50	50	50
16	Rice bran protein		-	50	50
Total			5000	5000	5000

Statistic Analysis of Data

To analysis obtained results from chemical, physical and sensory experiments, Minitab software has been applied. Applied statistical design in the present study had been absolutely random and each treatment included two iterations for (chemical, microbial and sensory tests), 4 iterations for (texture measuring test), and 3 iterations for (color measuring test). Obtained results from the design have been analyzed through one-way and tow-way ANOVA. Excel program has been also applied to trace diagrams. $P < 0.05$ has been also considered as significant difference among treatments.

RESULTS AND DISCUSSION

Operational Properties of rice bran Protein Concentrate

Nitrogen Solvability Index

After producing sample in pH ratios of 2, 4, 6, 8, 10, and 12, through applying HCl and NaOH, Nitrogen solvability index was measured using Bera and Mukherjee method. Obtained values have been presented in table4.

Research Article

Table 4: Nitrogen solvability of rice bran protein concentrate

Row	pH	Nitrogen solvability index of rice bran protein concentrate
1	2	37±0.72
2	4	4±0.64
3	6	9±0.23
4	8	56±0.81
5	10	64±0.93
6	12	62±0.75

According to table 4, nitrogen solvability rate in pHs of 2, 4, 6, 10, and 12 has been respectively equal to 37, 4, 9, 56, 64, and 62%. Accordingly, in higher and lower pHs than iso-electric point, nitrogen solvability rate of rice bran protein has been increased; where, in pH=10 it has been in maximum range and in pH=4, which is close to iso-electric point of rice bran protein concentrate, it has been achieved minimum rate.

Physicochemical and microbial properties of control sample and sausage treatments

Chemical test's results of produced sausage by rice bran protein concentrate;

Effects of replacing gluten and soya isolate with rice bran protein concentrate on chemical properties of samples have been presented in table 5.

- Total fat rate of control sausage was equal to 17.75% and in enriched sausage with 1% of rice bran protein concentrate replaced instead of gluten, was increased to 17.79%. In enriched sausage with 1% of rice bran protein, replaced instead of soya isolate, the fat ratio was increased to 17.83%. The ratios have shown respectively 0.22 and 0.45% total fat increase in final product.
- Total protein rate of control sausage was equal to 11.28% and in enriched sausage with 1% of rice bran protein concentrate replaced instead of gluten, was equal to 11.09%. In enriched sausage with 1% of rice bran protein, replaced instead of soya isolate, the protein ratio was decreased to 10.84%. The ratios have shown respectively 1.68 and 3.9% total protein reduction in final product. The difference has been more significant in treatment3 than control sample.
- Total moisture rate of control sausage was equal to 54.83% and in enriched sausage with 1% of rice bran protein concentrate replaced instead of gluten, was equal to 54.77%. In enriched sausage with 1% of rice bran protein, replaced instead of soya isolate, the moisture ratio was increased to 54.98%. The ratios have shown 0.10 % reduction in final moisture rate and 0.27% total moisture increase in final product. The difference has been more significant in treatment3 than control sample.
- Ash rate in control sample was equal to 2.52% and in treatments 2 and 3 the ash rate was respectively equal to 2.53 and 2.56%, which is not significant statistically different from control sausage.
- Total pH ratio of control sausage was equal to 6.12% and in enriched sausage with 1% of rice bran protein concentrate replaced instead of gluten, was equal to 6.13%. In enriched sausage with 1% of rice bran protein, replaced instead of soya isolate, the pH ratio was equal to 6.12%. Therefore, pH ratio in treatment 2 has been increased about 0.163%, comparing to control sausage, which the difference is not significant.

Chemical properties of produced sausages have been presented in table 5.

Research Article

Table 5: Chemical properties of produced sausages

Row	treatment	Factor	Fat (%)	Moisture (%)	Protein (%)	Ash (%)	pH
1	Formula No1 (control sausage)		a 17.75±0.05	a 54.83±0.10	a 11.28±0.14	a 2.52±0.02	a 6.12±0.007
2	Formula No2 (enriched sausage of 1% instead of gluten)		a 17.79±0.05	a 54.77±0.10	ab 11.09±0.14	a 2.53±0.02	a 6.13±0.007
3	Formula No3 (enriched sausage of 1% instead of soya isolate)		a 17.83±0.05	a 54.98±0.10	b 10.84±0.14	a 2.56±0.02	a 6.12±0.007

• **Color measurement test**

Effects of adding rice bran protein concentrate on color of produced sausages have been presented in table6.

Table 6: Effects of adding rice bran protein concentrate on color of produced sausages

Row	Treatment	Factor	b*	a*	L*
1	Formula No1 (control sausage)		a 6.84±0.05	a 5.09±0.007	a 28.58±0.05
2	Formula No2 (enriched sausage of 1% instead of gluten)		a 6.81±0.05	a 5.09±0.007	a b 28.50±0.10
3	Formula No3 (enriched sausage of 1% instead of soya isolate)		a 6.78±0.05	a 5.08±0.007	b 28.43±0.10

*L**, *a**, and *b** respectively present lightness of product, color tendency toward red, and color tendency toward yellow, comparing to control sample. In each column, values with different superscript letters are significantly different from each other equal to ($p < 0.05$).

Effects of adding rice bran protein concentrate on texture of produced sausages have been presented in table 7.

Table 7: Assessing texture of produced sample sausages

Row	Treatment	Factor	Shear force (N)	Required energy for shear (N. mm)
1	Formula No1 (control sausage)		a 17.54±1.01	a 128.98±16.38
2	Formula No2 (enriched sausage of 1% instead of gluten)		a 17.66±1.01	a 129.86±16.38
3	Formula No3 (enriched sausage of 1% instead of soya isolate)		a 17.35±1.01	a 126.91±16.38

Research Article

In each column, values with different superscript letters are significantly different from each other equal to ($p < 0.05$). According to table3, through applying rice bran protein concentrate in treatment 2, shear force and required energy for shear have been increased; although in treatment 3, shear force and required energy for shear has been decreased, comparing to control sample. Although the value is not significant, comparing to control sample. Therefore, adding rice bran protein concentrate has no negative effect on texture of samples.

Sensory Properties of Control Sample and Sausage Treatments

Some properties in table8 have been presented for sensory evaluation of produced treatments. Effects of adding rice bran protein concentrate on sensory properties of samples have been presented in table 8.

Table 8: Sensory properties of sample sausages

Row	Treatment	Factor	Color	Smell	Taste	Texture
1	Formula sausage)	No1 (control	a 5.65±0.11	a 5.20±0.05	a 5.15±0.05	a 5.05±0.10
2	Formula sausage of 1% instead of gluten)	No2 (enriched instead of	a 5.59±0.11	a 5.16±0.05	a 5.18±0.05	a 5.01±0.10
3	Formula sausage of 2% soya isolate)	No3 (enriched instead of	a 5.50±0.11	a 5.28±0.05	a 5.21±0.05	a 5.18±0.10

In each column, values with different superscript letters are significantly different from each other ($p < 0.05$). In this study, control sausage is not significantly different from treatments 2 and 3 in regard with evaluation factors and rice bran protein can be replaced easily instead of gluten or soya isolate.

Microbial Properties of Control Sample and Sausage Treatments

Table 9: Microbial properties of control sample and sausage treatments

Treatment	Total No	Coliform	E. coli	Salmonella	Staphylococcus aureus	Clostridium Prem Franz	Mold and yeast
Standard	10^5	10<	Negative	Negative	10<	50	102
Formula 1	3.4×10^4	Negative	Negative	Negative	10<	10<	10<
Formula 2	3.4×10^4	Negative	Negative	Negative	10<	Negative	10<
Formula 3	3.5×10^4	Negative	Negative	Negative	Negative	10<	10<

Conclusion

Sensory evaluation of treatments has indicated that, treatment2 (enriched by 50gr/5kg rice bran protein concentrate instead of gluten) and treatment3 (enriched by 50gr/5kg rice bran protein concentrate instead of soya isolate) were not significantly different from control sample statistically. Therefore, considering obtained results from statistical analysis of sensory properties of sausages, it could be mentioned that, sensory properties of treatments2 and 3 were similar to sensory properties of control sample. Successful results of improvement of texture properties indicated that applying 50gr/5kg rice bran protein concentrate instead of soya isolate could lead to improvement and smoothness of sausage, comparing to

Research Article

control sausage. Using rice bran protein concentrate caused also no significant difference in pH ratio. Regarding obtained results from statistical analysis of chemical, physical and sensory evaluations of samples with rice bran protein concentrate, it could be mentioned that applying the rice bran protein concentrate in formulation of produced sausages has not lead to significant difference in them, comparing to control sausage. Therefore, the rice bran protein concentrate could be replaced instead of gluten or soya isolate.

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Research Article

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