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Health benefits of soy proteins analysis and their influence on the quality of fortified bread

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Abstract

Aliments don't contribute only to health maintenance and an optimal development, but they can have an important role in reducing the risk of getting sick. Bread fortified with soy proteins has a benefic effect for health, in preventing sickness, especially the proteic malnutrition and coronary heart disease being recommended to big groups of individuals at whose level an unequivocal insufficiency in proteins is detected, as well as an inadequate and unbalanced contribution in essential amino acids. This study shows the evaluation of the quality of bread products fortified with soy degreased flour that will be made by calculating the chemical value of limitant essential amino acids and of the essential amino acids EAA-Index. An increase of all essential amino acids content was observed with the concentration increase of used protein addition

Keywords: functional food, health benefits, bioactive compound, soy protein, fortified bread, quality

1. Introduction

While food has long been used to improve health, knowledge of the relationship between food components and health is now being used to improve food. Food and nutrition science has moved from identifying and correcting nutritional deficiencies to designing foods that promote optimal health and reduce the risk of disease. Functional foods provide essential nutrients often beyond quantities necessary for normal maintenance, growth, and development, and/or other biologically active components that impart health benefits or desirable physiological effects. Functional foods can provide health benefits by reducing the risk of chronic disease and enhancing the ability to manage chronic disease, thus improving the quality of life.

Functional foods also can promote growth and development and enhance performance.

Building a strong scientific basis for functional food claims relies on the ability to demonstrate the efficacy of the food's bioactive component(s). Demonstrating efficacy in experimental animals, while not trivial, is quite straightforward. Proving efficacy in humans is substantially more difficult. Most of the epidemiological associations of diet and reduced disease risk relate to overall dietary practices, not a single bioactive component. Linking specific benefits to the consumption of individual foods or specific food components is difficult and requires rigorous scientific protocols [10].

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Dietary *trans*-unsaturated fatty acids can increase plasma LDL and reduce HDL cholesterol concentrations. Diets low in SFAs and *trans*fatty acids could therefore reduce the risk of cardiovascular disease. The *cis*-unsaturated fatty acids with 18 carbon atoms – oleic (mono-unsaturated), linoleic and alpha-linolenic acids (polyunsaturated) – reduce plasma concentrations of LDL cholesterol and some may also raise plasma concentrations of HDL cholesterol. Functional foods enriched in these unsaturated fatty acids could also be used to reduce the risk of cardiovascular disease. Like alpha-linolenic acid, the long-chain, highly unsaturated PUFAs found in fish oils belong to the n-3 family. They can promote improvements in endothelial and arterial integrity as well as counteract blood clotting and reduce blood pressure. They also reduce plasma TAG levels and may have suppressive effects on the cellular immune system. One of the focus areas of functional food development concerns the incorporation of n-3 fatty acids into foods [6].

2. Materials and methods

2.1. Criteria used in functional food's efficacy evaluation: Hill (1971) proposed specific criteria to use in evaluating research findings, and these criteria have guided the evaluation of diet and health interrelationships for the last two decades. Some structure/function claims for specific foods have been successfully developed and supported by FDA by following these criteria. However, the process has been hampered by limitations in the current regulations and/or government interpretations of those regulations.
Hill's Criteria are presented in the following:

Strength of association – how statistically significant and convincing are the data that support the relationship?

Consistency of the observed association – how well do the available data from different sources, areas, and types of studies support the relationship?

Specificity of the association – do the data demonstrate a predictable relationship between the bioactive component and the proposed effect?

Temporal relationship of the observed association – is the proposed effect observed following treatment with the bioactive component?

Dose-response relationship – do the data demonstrate a magnified effect of the bioactive component with increasing dose?

Biological plausibility – is there a plausible mechanism to explain the effects of the bioactive component?

Coherence of the evidence – does the relationship help explain the available data, when viewed as a whole? [5, 11].

In applying the Hill criteria to the research findings, the IFT Expert Panel believes it is also necessary to consider:

The amount and type of evidence – The amount and type of evidence sufficient to demonstrate efficacy will vary for each functional food component. All forms of competent and reliable scientific research are considered. As a rule, well controlled human clinical studies are the most directly applicable and understood form of evidence and therefore are given the most weight. When a clinical study is not possible, epidemiological evidence may be considered, such as research explaining the biological mechanism underlying the proposed effect. Animal *in vivo* studies are useful, particularly where they are widely considered to be acceptable substitutes for human research or where human research is not feasible. Animal *in vivo* studies also can be used to determine the underlying mechanisms by which the functional food produces its effect. Although no specific number of studies can be set, the replication of research results in an independently conducted study adds to the weight of the evidence.

Quality of evidence – The quality of a study is paramount; evidence for reproducibility and internal validation of quality are critical. The design, implementation, and analysis of results must be conducted in a competent and reliable manner following accepted principles for testing hypotheses. General principles accepted in the scientific community to assure the validity of studies for demonstrating efficacy include: (1) carefully controlled double-blind structure; (2) sufficient duration to establish long-term efficacy; (3) appropriate dosing regimens to document a dose-response relationship; (4) recognized biological or chemical mechanism/biomarker that explains the effect; (5) statistical significance of findings; and (6) results that provide a meaningful benefit for consumers.

Where one or more of these cannot be met, qualified experts must consider the impact on the conclusions.

The totality of the evidence – Studies cannot be evaluated in isolation, and all relevant research should be considered. In fact, the context of the scientific evidence is just as important as the internal validity of individual studies. The studies used to substantiate a claim must be largely consistent with the surrounding body of evidence. Wide variation in study outcomes and inconsistent or conflicting results will raise serious questions about efficacy. Inconsistencies in the evidence must be examined to determine whether plausible explanations exist. In some cases, different results are attributable to differences in dosage, the form of administration, the population tested, or other aspects of study methodology. The contribution of non-dietary factors such as smoking, and environmental contaminants or conditions may need to be evaluated.

The relevance of the evidence to the specific claim –Research supporting efficacy claims must be relevant to both the food product and the specific benefit being claimed. Necessary questions include: How does the dosage and formulation of the proposed functional food product compare with that used in the study? Does the product contain additional ingredients that might alter the effect of the functional ingredient? Is the product administered in the same manner as the ingredient used in the study? Does the study population reflect the characteristics and lifestyle of the target population? If research conditions differ significantly from the use being promoted, additional research may be needed to support extrapolation from study results to claimed effect [10].

The IFT Expert Panel recommends that evaluation of a functional food's efficacy rely on the Hill criteria. These evaluations must explicitly address the strength and relevance of the data supporting the bioactive component's specific role in improving the health outcome of interest.

*2.2. Materials and methods used in quality analysis of fortified bread.*The flour used in these experiments was white flour, type 650, obtained by means of grinding wheat of Triticum aestivum L species, in the mill of S.C. Boromir Prod Buzău.

In order to standardize the content of α-amylase, the flour was ameliorated in the mill by adding 1g α-amylase/100 kg flour, with an amilolitical activity of 140000scp/g. In order to increase the extensibility of the dough, to decrease the unfavorable effect of the insoluble hemicelluloses on the continuity of the gluten motion and to increase the conservation period of bread freshness, 6g hemicellulase/100 kg flour have been added. For a better tolerance of the dough rising, 2g xilanase/100 kg flour has been added, and for the hardening of the gluten receipt 1g ascorbic acid/100 kg flour has been added. In order to improve the technological quality of the flour 8 g cystein (50%)/100 kg flour have been added.

The protein additions used in this study was soy degreased flour without enzymatic activity (PROVABIS), whose main physical and chemical parameters are indicated in table 1. The exogenous protein was added into witness flour in different concentrations of 3, 5, 10 and 15%, studying the influence of those additions on the protein value of bread.

Table 1. Chemicals parameters of soy degreased flour

Component	U.M.	Soy degreased flour without enzymatic activity
Proteins	%	min 52
Fat	%	+/- 1,5
Ash	%	max 7
Moisture	%	8

The fortified products were assessed by carrying samples through the indirect baking process (SR ISO 6820:2002 and SR 91-83). The purpose of these tests was to determine the influence of added protein on the sensory characteristics of the product, on volume, elasticity, porosity and acidity of it. On the other hand it sought to increase the proteic value of bread while increasing the concentration of added protein used, the proteins quality, in order to determine to what extent it can optimize the bread quality while increasing the protein content of it.

For a good comparative appreciation of the effects of the proteic supplements over the organoleptic characteristics of the bread, their sensorial evaluation was realized through scoring, using an evaluation scale of 20 points. The quality evaluation of the proteins consisted in the analysis, by liquid chromatography of high performance (RP-HPLC), of bread samples obtained from the fortification of wheat flour with sodium caseinate (Altmann, 1992).

A Hewlett-Packard HP 1050 series system equipped with a programmable auto injector capable of performing the pre column derivation step was used.

3. Results and discussion

Food has traditionally been viewed as a means of providing normal growth and development. Regulatory policies were established to replace nutrients lost during processing and, in some cases, to prevent nutrient deficiencies in the population.

3.1. Efficacy evaluation of soy protein **-** Soy protein has been shown in numerous trials to reduce serum cholesterol in men and women with mild to moderate hypercholesterolemia. Thus, a meta-analysis consisted on 38 studies concluded that substituting soy protein (from isolated soy protein (ISP) or from textured vegetable protein) for animal protein significantly lowered total and LDL cholesterol and triglycerides, without affecting HDL cholesterol [1]. Over the next few years, a number of larger, placebo-controlled, clinical trials verified these conclusions. In one study, mildly hypercholesterolemic men consumed up to 50 grams/day of soy protein, and a dose-response relationship was noted when comparing serum cholesterol reduction after 3 and 6 weeks of feeding [9].

Many researchers have suggested that the isoflavones are responsible for lowering lipid levels. In a 9-week study of both men and women fed with 25 grams of ISP daily with varying amounts of isoflavones, was found that only ISP diets with higher amounts of isoflavones depressed serum cholesterol [3]. However, removing isoflavones by alcohol washing the soy protein also removes other bioactives such as saponins that may affect lipid metabolism, so the role of isoflavones is difficult to measure [4]. The isoflavone-rich ethanol extract from soy has not been shown to significantly reduce serum cholesterol, although this fraction may have direct positive effects on the vascular system, such as improving systemic arterial compliance [7]. Therefore, some synergy among the components of intact soy protein appears to provide the maximum hypocholesterolemic properties [4].

FDA reviewed the strength of the relationship between soy protein and lipid reduction.

When considered in the context of the Hill criteria, the evidence evaluated by the Agency demonstrates that the Hill criteria were generally satisfied (Table 2).

Although the mechanism of action and the exact bioactive components responsible for cholesterol reduction were unknown, FDA nevertheless approved a health claim for the lipid-lowering capabilities of soy in 1999 [10].

3.2. The influence of soy protein addition on the quality of fortified bread - As expected the protein contribution of bread fortified with soy degreased flour registered a growing evolution with the increase of the quantity of used proteic addition. The content of all amino acids increased with the increasing of the quantity of used protein addition. To analyse the efficiency of bread fortifications with soy degreased flour, the amino acid content of the fortified samples, expressed like dried substance, was compared to the essential amino acids content of FAO protein.

It can be observed that the limitant amino acids of the samples fortified with sodium degreased flour remain the lysine and the threonine, although their deficit decreases through fortification. So the covering proportion of the lysine, compared to FAO protein, increases from 32,64%, according to the unfortified sample, to 56,44%, according to the sample fortified with 15% soy degreased flour (table 4). Taking into account the maximum admitted technological limit, namely the fortification with the maximum of 10% soy degreased flour, the covering proportion of lysine, compared to FAO protein, cannot exceed the value of 53,80%. The covering proportion of the threonine, compared to FAO protein, increases from 77,25%, according to the unfortified sample, to 92,13%, according to the sample fortified with 15% soy degreased flour, the maximum fortification technologically admitted, with 10% soy degreased flour, limiting this increase to 87,00 % (table 3).

The chemical values have the inconvenient that they do not permit the nutritional availabilities of dozed amino acids to be known. The „Chemical Score” represents the proportion between the content of the limitant essential amino acid of the tested protein biological value and the content of the suitable amino acid from the proper protein [8]. The chemical values of bread products fortified with sou degreased flour are presented in table 4.

The obtained results indicate an increasing variation of all the values of lysine chemical ratings with the growth of the concentration of soy degreased flour. Thus, the fortification assures an increasing variation of the chemical value of lysine with the growth of protein addition used, between 33,51%, which corresponds to a protein addition of 3%, and 56,44%, that corresponds to a fortification with 15% exogenous protein. The bread fortified with 10% degreased soy flour (the biggest technological limit admitted) are also the ones that our body needs, to maintain the nitrogenous balance sheet, with a chemical value of lysine of 53,80%.

The chemical value of treonine, the second essential limitant amino acid of bread products, showed increasing values with the growth of the concentration of the protein addition used.

The values of this chemical rating varied between 82,55-87% according to the concentration of the exogenous protein used at fortification, these values proving that the contribution of treonine in fortified products assures, no matter the situation, the maintaining of the nitrogenous balance sheet of the body. The chemical values of essential amino acids of bread products fortified with soy degreased flour are indicated in table 6. The biological value of a protein is influenced not only by their limitant amino acids but by the deficit of other essential amino acids, and because of that, it is recommended the reckoning of the value of essential amino acids (EAA – Index), that it is based on the ensemble of all the essential amino acids (table 5). Degreased soy flour assures the quantity needed by the essential amino acids, as well as the right balance for those, superior to the one corresponding to the other protein sources [2].

Table 2. Soy proteins and coronary heart disease Source: (IFIC Foundation, 2007)

Hill criteria	Evidence supporting/Against
Biologic gradient: evidence of a dose-response curve	"The intervention studies suggest that a minimum level of approximately 25 g of soy protein is needed to have a clinically significant effect on total and LDL cholesterol levels." "FDA agrees that the available data on the hypocholesterolemic effects of soy protein do not permit a dose-response assessment. However, FDA notes that dose-response data are not required to establish the qualifying criteria for a substance that is the subject of a health claim."
Plausibility: association is biologically plausible	"Other comments reviewed various possible mechanisms for the cholesterol-lowering effects of soy protein and some argued that until the mechanism of action of soy protein is clearly established, no health claim should be authorized. FDA notes, however, that such knowledge is not necessarily required for authorization of a health claim." "The evidence shows a clear relationship between soy protein and reduced risk of CHD despite lack of a clearly defined mechanism for its effect."
Coherence of explanation: association is consistent with current knowledge of the disease/endpoint and biomarkers known to be associated with it	"It is generally accepted that blood total and LDL cholesterol levels can influence the risk of developing CHD, and, therefore, that dietary factors affecting these blood cholesterol levels affect the risk of CHD."
Strength of association: a strong association is less likely to be the result of errors	"In most intervention trials in subjects with mildly to moderately elevated cholesterol levels (total cholesterol <300 mg/dL), plant sterol esters were found to reduce blood total and/or LDL cholesterol levels to a significant degree."
Consistency upon repetition: association has been observed by different persons in different places, circumstances, and times	"Four studies show a relationship between consumption of plant sterols and reduced blood cholesterol in hypercholesterolemic." "The results of three studies support a cholesterol lowering effect of plant sterols in subjects with normal cholesterol values." "Two studies showed a relationship between consumption of plant stanol esters and reduced blood cholesterol in hypercholesterolemic subjects who consumed plant stanol esters as part of a low saturated fat and low cholesterol diet." "Eight studies show a relationship between consumption of plant stanols and reduced blood total and LDL cholesterol in hypercholesterolemic. Two studies show a relationship between consumption of plant stanols and reduced LDL cholesterol, but not blood total cholesterol." "Two studies show a relationship between consumption of plant stanols and reduced blood cholesterol in subjects with normal cholesterol concentrations."
Specificity: a specific association is evidence in favor of causality	"Given the variability of amounts and of food carriers in which plant sterols and plant sterol esters were provided in the diets studied, the response of blood cholesterol levels to plant sterols appears to be consistent and substantial, except for plant sterols from sheanut oil and ricebran oil."

Table 3. The covering proportion of the essential amino acids content of FAO protein by the samples fortified with soy degreased flour

Proteic addition [%]	The covering proportion of FAO protein content, [%]							
	Thr	Tyr +Phe	Val	Ile	Leu	Lys	Cys + Met	Trp
Soy degreased flour								
control	77,25	154,05	97,48	105,73	120,26	32,64	124,66	121,10
3%	82,55	155,60	97,90	106,53	120,53	33,51	125,31	121,90
5%	83,58	155,63	98,28	106,95	120,61	40,25	125,49	123,10
10%	87,00	155,65	98,60	107,15	120,93	53,80	151,89	124,60
15%	92,13	155,78	98,82	107,48	121,20	56,44	152,83	124,90

Table 4. The chemical values of bread products fortified with different semi-conventional proteins

Chemical value, [%]	The size of the semi-conventional exogenous addition				
	control	3%	5%	10%	15%
Soy degreased flour					
$I_{c\ Lys}$	32,64	33,51	40,25	53,80	56,44
$I_{c\ Tre}$	77,25	82,55	83,58	87,00	92,13

Table 5. Chemical values of essential amino acids of bread products fortified with soy degreased flour

Protein addition	EPV_{Lys}	EPV_{Tre}	$EPV_{Tyr+Phe}$	EPV_{Val}	EPV_{Ile}	EPV_{Leu}	$EPV_{Cys+Met}$	EPV_{Trp}	EAA-Index
Soy degreased flour									
control	32,64	77,25	154,05	97,48	105,73	120,26	124,66	121,10	96,09
3%	33,51	82,55	155,60	97,90	106,53	120,53	125,31	121,90	97,65
5%	40,25	83,58	155,63	98,28	106,95	120,61	125,49	123,10	100,31
10%	53,80	87,00	155,65	98,60	107,15	120,93	151,89	124,60	107,33
15%	56,44	92,13	155,78	98,82	107,48	121,20	152,83	124,90	108,99

4. Conclusion

Several food components may provide additional dietary options in the prevention and treatment of cardiovascular heart disease. Soy protein can reduce serum cholesterol in men and women with mild to moderate hypercholesterolemia. Several intervention studies suggested that a minimum level of approximately 25 g of soy protein is needed to have a clinically significant effect on total and LDL cholesterol levels. The scientific evidence shows a clear relationship between soy protein and reduced risk of cardiovascular heart disease despite lack of a clearly defined mechanism for its effect.

An increase of all essential amino acids content was observed with the concentration increase of used protein addition, fact that can be noticed through the growth of the values of the chemical values of all the essential amino acids. We can observe that the limiting amino acids of the samples fortified with soy degreased flour remain the lysine and the threonine, although their deficit decreases through fortification.

By examining the amino acids content of samples fortified with soy degreased flour corresponding to the maximum technologically admitted concentration, we can observe the fact that lysine deficit was partial recovered.

Consumers might select functional foods and tailor their diets to meet changing health goals and different requirements at different ages. Future benefits might include functional foods for increased energy, mental alertness, and better sleep.

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