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CONTENT

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COMPARATIVE EFFECTS OF ESSENTIAL OILS ON GROWTH OF *Escherichia coli*

Vesna Kalaba[1*], Dragana Kalaba[2]

*[1]PI Veterinary Institute of the Republic of Srpska "Dr. Vaso Butozan" Banja Luka, Republic of Srpska, Bosnia and Herzegovina; * vesna.kalaba@yahoo.com*
[2]Faculty of Medicine-Department of Pharmacy, Banja Luka, Republic of Srpska, Bosnia and Herzegovina

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ABSTRACT
This paper examines the antimicrobial activity of essential oils: anise, fennel, mint, orange, cinnamon, eucalyptus and rosemary on the growth of *Escherichia coli*. Different concentrations of essential oils were prepared by dissolving with 96% ethanol (1:1 and 2:1) and their antimicrobial effect on growth of *E. coli* was determined by disk diffusion method. Essential oil of anise and lemon did not show any antimicrobial activity, while inhibitory action of others varies depending on the type of plants and oil concentration. Essential oil of cinnamon, thyme, fennel and cloves showed bactericidal effect, while essential oil of rosemary, mint, eucalyptus and orange showed bacteriostatic effect.

1. Introduction

Microorganisms frequently lead to food spoilage, so their presence is one of the biggest problems that we have to face. A wide range of microorganisms, such as *Escherichia coli, Staphylococcus, Campylobacter, Listeria monocytogenes, Salmonella spp.,* agents of food spoilage and disease are transmitted by food eating. For this reason it is necessary to use chemical preservatives as a preventive measure for the growth of microorganisms in the food production process (Sagdic and Ozcan, 2003). However, today, more attention has to be paid to the implementation of components of natural products isolated from plant products (Hsieh et al, 2001). Consumers and producer express their interest in the application of essential oils from aromatic plants with antimicrobial activity to the control and management of pathogenic and/or toxic microorganisms in food (Valero et al., 2003; Soliman et al., 2002). Such interest is justified because there are estimations according of over 30% of the population each year in industrialized country suffers from foodborn diseases and in 2000 at least 2 million people died of diarrhea worldwide (Clarke, 2001, WHO-World Health Organization, 2002). A way of overcoming this problem is the constant development of new applications of available natural antimicrobial substances (Skocibusicet al., 2006).

Since ancient times was known spice and medicinal plants show different antimicrobial activity. More than 1340 plants are known as a potential source of antimicrobial components, but the small number are studied in detail (Wilkins and Board, 1989). From natural plant ingredients the flavonoids, saponins, tannins and alkaloids are known to have antimicrobial activity. The antimicrobial activity of plants such as thyme, rosemary, orange, clove etc., comes from their essential oils. Essential oils are easily volatile, aromatic substance, naturally present in plant life, especially in every plant with strong aroma. They can be founded in all parts of plants or can be concentrated in one part of it (flowers, leaves, seeds, roots, bark). The amount of essential oils in herbs varies within wide limits. In some plants they are present in very small amounts (0.05-0.1%)

while in other they are up to 20% (clove) (Popovic and Djurdjevic Milosevic, 2008). Essential oils own natural biologically active substance with insecticidal and antimicrobial activity, which are very important in the food industry for preserving food and preventing growth of pathogenic microorganisms. As result, the herbs or their essential oils have found application in the food industry as food additives that are used to achieve the appropriate sensory characteristic, but which at the same time can provide adequate stability and safety of food. In Western countries, the food industry respondents think that the antimicrobial properties of herbs have limited value and thus they should be added in too large quantities before they get certain results. As result, the consumers consider such food too spicy.

Escherichia coli is a Gram-negative bacteria, facultative anaerobic and very resistant that adapts easily to different conditions in the environment. It is very common contaminant of various food products (e.g. milk and milky products, meat and meat products, water, insufficiently washed fruits and vegetables) (Buchanan and Doyle, 1997).The most common way of transmission of this bacteria is from person to food and from food to person. Diseases caused by *Escherichia coli* have always related to poor hygiene and living conditions and food preparation (Bem, 1991). Microbiological food safety can provide adequate application of pasteurization and cooking food and subsequently, the prevention of products' contamination. Growth control of *E. coli* is one of the major goals in the processing industry and food production. Strains of *E. coli* and related Gram-negative coliforms bacteria are the dominant flora in the komensal intestine of man and animals. Species of *E. coli* contain a number of strains which include clean komensal organisms, but also those who have a combination of virulence determinants that allow them to act as specific pathogens of the digestive tract and other systems, particularly urinary (Koneman, 1997). Among strains, *Escherichia coli* are particularly important by those who have the ability to produce certain toxic substance, which cause different diseases in humans. Intestinal diseases have often caused an epidemic among infants and young children, and adults. Enterotoxic and enteropathogenic *Escherichia coli* are most important when it comes to outbreaks of diarrhea among the human population (Schmidt i sar.1997). Enterotoxic *E.coli* produces thermolable or thermostable enterotoxin that causes secretory diarrhea, similar to that caused by *Vibrio cholera* (Koneman, 1997). This disease is usually treated with appropriate antimicrobial substances, but such treatment is often not efficient because of the existence of resistant strains (Cid et al., 1996). This is why it is necessary to find antimicrobial substances which operate more efficiently than those currently in use.

In this line, the aim of this study is to determine the antimicrobial activity of essential oils of different plant species (anise, fennel, mint, orange, cinnamon, eucalyptus and rosemary) on the growth of *Escherichia coli.*

2. Materials and methods

To investigate the effect of essential oils on the inhibition growth of *Escherichia coli,* the diffusion method was used. The culture of *Escherichia coli* ATCC 25922 sown in nutrient broth and incubated at 37^0C was used. Petri plates with the appropriate substrate were inoculated with 0.1 ml bacterial suspension in concentration of 10^9 CFU/ml. The surface of Petri plates prepared under sterile conditions, were put on sterile cylinders previously instilled by micropipette with 10 µl of appropriate essential oil and then incubated for 24 hours at 37ºC.

Essential oil was used as pure, mixed with 96% alcohol in the proportion 1:1, and as combinations mixed with alcohol in the ratio of 2:1. As a control, the disc is instilled with clean 96% alcohol volume of 10 µl. Three replicas were performed for each essential oil. After 24 h incubation, the results were red by measuring the inhibition zone diameter and expressed as average.

The type of action (bactericidal or bacteriostatic) of each essential oil was established. Thus, a small piece of agar from inhibition zone was extracted, added to the nutrient broth and incubated for 24 h at 37°C. If after incubation the blur broth occurs, it was considered

as a bacteriostatic effect of essential oils. Otherwise, if the broth remained clear, the effect of the oil was considered as bactericidal.

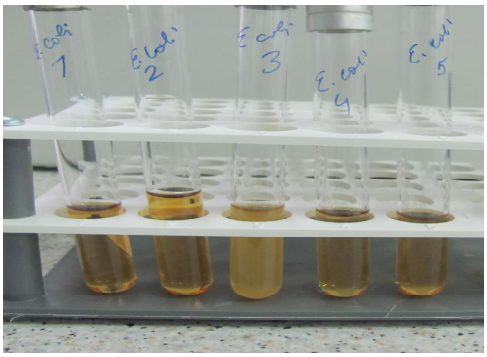

Figure 1. Display of bacteriostatic and bactericidal effects of essential oils against *E. colli*

3. Results and discussions

The results obtained by examining the effects of essential oils against the growth of *Escherichia coli* are presented in Table 1. The impact of essential oils against *E. coli* varies in a wide range. The strongest growth inhibition is displayed by the essential oils of cinnamon, rosemary, thyme, clove and mint, but activity of essential oils of orange, fennel and eucalyptus is not negligible. The essential oils of anise and lemon had no effect on the growth of *Escherichia coli.* Pure essential oils show a stronger effect on the growth of *Escherichia coli* in relation to the effects of essential oils in combination. From type of action point of view, was found that the essential oils of cinnamon, thyme, fennel and cloves displayed bactericidal, while essential oils of rosemary, mint, eucalyptus and orange showed the bacteriostatic effect. Our results are consistent with the works of other authors (Dorman and Deans, 2000; Burt and Reinders, 2003; Popovic and Milosevic, 2008; Miletic et al., 2009; Kalaba et al., 2013).

4. Conclusions

Based on experimental data, the next conclusions can be drawn: (i) With the exception of anise and lemon, the rest of essential oils showed certain antimicrobial activity. The difference depends on the oils sources and concentration (ii) The essential oil of cinnamon, rosemary and thyme displayed the strongest antimicrobial activity; (iii) Essential oils of cinnamon, thyme, fennel and cloves showed bactericidal effect while essential oils of rosemary, mint, eucalyptus and orange showed bacteriostatic effect.

Individual or in a combination, the essential oils can provide an effective action against to *Escherichia coli*, which achieves adequate guarding and preserving of food.

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MULTIMODAL DISCOURSE ANALYSIS OF INTERACTIVE MEANING OF FOOD ADVERTISEMENT PRINTED IN ENGLISH

Jing Wang[*1], Yanli Dai[2], Lan Jiang[1]

[1]*Hebei Normal University of Science and Technology, Qinhuangdao, Hebei, 066000, China; * wjdyljl@163.com*
[2]*Hebei Institute of Foreign Languages, Qinhuangdao, Hebei, 066311, China*

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ABSTRACT
Analysis of traditional advertisement printed in English basically focuses on language rather functions of non-linguistic symbol resources play in discourse meaning construction. The latter emerging multimodal discourse analysis breaks this limitation to a large extent. This paper analyzed a piece of multimodal advertisement discourse with system-functional linguistics of Hal Hday and visual grammar of Kress and Van Ixeuwen as theoretical framework. It is found that, language and image, the two symbol resources, form join forces by mutual strengthening and supplement, with their own unique means, jointly create overall meaning of discourse and achieve the best persuasion effect.

1. Introduction

American Marketing Association defines advertisement as a kind of public information exchange activity, and advertiser introduce product, services or concept to public through various communications media by means of paying (Mei, 2010). We can see that, the main interaction purpose of advertisement is to persuade public to purchase some product or service, accept some concept or adopt some behavior by means of rational appeals and emotional appeals. Generally speaking, the guiding thoughts of advertising idea include object-oriented (product) and people-oriented (customer). Successful advertisement depends more on the satisfaction of psychological demand of people (Gang, 2012). Previous advertisement discourse basically focuses on copy, and emphatically studies vocabulary, grammar, text and rhetorical features (Zi'ang, 2013).

Multimodal discourse emerged in the 1990s breaks through this limitation to a large extent. Taking a piece of multimodal food advertisement discourse on American Reader' Digest as an example, this paper attempted to explore how language and image jointly construct overall meaning of discourse by their own unique means under the framework of system-functional grammar and visual grammar, in order to achieve scheduled interactive purpose.

2. Materials and methods

2.1 Materials

The discourse is the advertisement of Maxwell House from American Reader's Digest (6) in May, 2009. As shown in Figure 1, this discourse is composed of image (image 1 and 2) and copy (title, main body, slogan).

2.2 Method

According to two identification criteria on multimodal discourse proposed by Zhu Yongsheng: (1) the amount of involved modal category; (2) the amount of involved symbol system, this discourse only involves visual modal, but it contain both words and images, that is, two kinds of symbol system, therefore, it belongs to multimodal discourse.

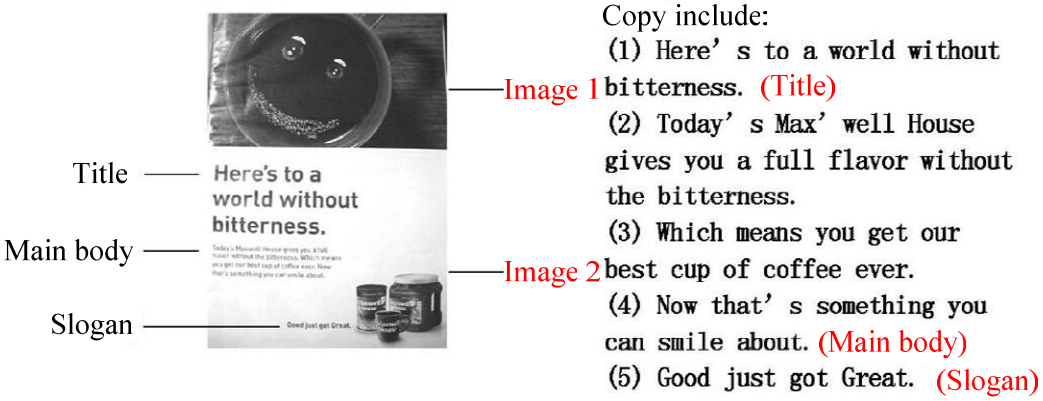

Figure 1. Max House Coffee Advertisement on Reader's Digest

Because of many components in the discourse, multimodal discourse cluster division of Baldry and Thibault is adopted for analysis. The discourse is divided into three clusters: Cluster 1 (image 1), Cluster 2 (title and main body) and Cluster 3 (image 2 and slogan), that is, the components that are close in position and correlated in function are classified into one cluster. Three clusters are analyzed respectively and then the overall meaning of discourse is constructed. During analyzing the ideational (represent) function and interpersonal (interactive) meaning, the system-functional grammar or visual grammar theory are applied. As for construction meaning, Kress and Van Leeuwen held that, discourse should be regarded as a whole rather in analysis. In additional, as mentioned above, the composition function of image is also suitable for analyzing multimodal with text and image mixed. Therefore, this paper applies composition function theory of visual grammar to analyze the whole discourse, that is, the composition meaning of three clusters. In summary, ideotional (represent) and interpersonal (interactive) meaning is first analyzed applying system-functional grammar and visual grammar theory and then the composition meaning of the whole discourse, finally the whole meaning of discourse construction.

2.3 Multimodal discourse analysis method and its theoretical basis

Since Harris proposed the research direction of discourse analysis in 1950s, discourse analysts worldwide began to dedicate to put forward various discourse theories and methods to analyze language system and semantic structure and their relationship with social culture and psychological cognition. However, these researches are all limited to language itself, but ignore or weaken other meaning resources, such as image, voice, color, animation. Therefore, it leads to the limited perspective on discourse function and meaning (Jinying, 2014; Yongsheng, 2007). Multimodal discourse analysis breaks through this limitation and promotes the organic combination of traditional and single language communication means with vision, hearing and gesture, thus forms two trends of modern discourse: image turn and multimodality. Multimodal means that, people use different symbol resources to perform meaning construction in social cultural situation (Zhanzi and Danyun, 2012). Therefore, multimodal discourse integrated multiple symbol resources to construct meaning (Zhang, 2009).

The main theoretical basis of multimodal discourse analysis is system-functional linguistics created by Halliday which holds that language is a social symbol with meaning

potential and multifunctionality, that is, it has functions of ideational function, interpersonal function and discourse function at the same time. Ideational function represents objective world and inner world; interpersonal function represents the relationship between speaker and listener and the attitude and implementation method of speaker to the content. In the specific social cultural field, same meaning usually can be expressed by different symbols; linguistic and non-linguistic modal has the common meaning potential; the semantics constructed by modals can be mutually compared. Therefore, the analysis framework of multimodal symbol characteristics often takes semantic as reference, such as process, mood, information value, etc (Zhanzi and Danyun, 2012). Till now, system-functional linguistics has been applied into the explanation of other symbol systems, such as researches on visual image, voice, architectural design, movie and website. Among them, the research of Kress and Leeuwen is the most representative (Qinhong, 2008). Based on the system-functional linguistic theory of Halliday, they extended idea of metafunction to visual model, and created visual grammar with representation function, interaction function and composition function as core to analyze the content. Representation function is corresponding to ideational function, and is used for expressing the objectives in the world and their relationship with each other. Kress and Van Leeuwen held that, language and visual interaction form same cultural meaning system by their own unique means. However, they have advantages and limitations. Some meaning can only be expressed by language and some only by image. In multimodal discourse, these two symbol resources can mutually strengthen (express same content in different means), supplied or be placed by grade (one symbol dominates and the other one adds meaning) to cooperatively fulfill interaction purpose.

Theoretical framework comparison of system-functional grammar of Halliday and visual grammar of Kress & Van Leeuwen is shown in Table 1.

In comparison, multimodal research of Chinese linguistics field is still in starting stage (Zhang, 2009). Since Zhanzi (2012) introduced this theory, some scholars such as Yongsheng (2007), Xinren (2009), Xing (2012), Qinhong (2008), Huabing (2013) and Zhang (2009) explored and studied it. The amount of published paper on it is increasing year by year, and multimodal discourse has gradually become a research hotspot. However, these researches focus on theoretical discussion or image analysis, and pay little attention on how words and image jointly construct meaning in context as well as the construction of multimodal advertisement discourse meaning. This paper attempted to construct the whole meaning of a piece of multimodal advertisement discourse for discussion.

3. Results and discussions

3.1 Meaning construction of Cluster 2

Participator establishes contact with reader, dominantly acknowledges reader's existence, implements image behavior of demand, seeks product and service from reader when he directly look at readers. If participator does not look at reader, then it expresses offer, that is, offer information. In figure 1, the personalized coffee contacts with eyesight of readers to express seek, to ask for establishing relationship with readers and smile stands for asking for establishing emotional relationship.

Attitude is represented through perspective, expressing the objective and subjective attitude of participator. To make interaction successful, participator should express clear information and select the expression means that is easy for understanding. In figure 1, coffee shows front perspective, which makes reader into it. Its face is at the eye level of reader, which symbolizes sense of identity that is at the same level of reader.

Modality refers to the truth and reliability of statement on the world.

Table 1. Theoretical framework of system-functional grammar and visual grammar

Symbol system	Language		Image	
Function	Concept	Transitivity (substance, psychology, relationship, behavior, speech, existing process); ergativity	Representation	Narrative representation (action, response, relationship, speech, psychology, transformation process), ideational representation (classification, analysis and symbolic process)
	Interpersonal	Mood, modality, person, assessment	Interaction	Contact, social distance, attitude and emotion
	Discourse	Theme, information, connection	Composition	Information value, salience, framing

Table 2. Transitivity analysis of Maxwell House advertisement copy

Sentence	Process type	Process	Participator
(1)	Relationship	is	Here, to a world without bitterness
(2)	Material	gives	Today's Maxwell House, you, a full flavor without the bitterness
(3)	Relationship	means	Which, you get our best cup of coffee ever
	Material	get	You, our best cup of coffee
(4)	Relationship	is	That, something you can smile about
	Behavior	Can smile	You

Judgment criteria are modality mark and coding orientation. Modality mark include color saturation, color discrimination, color coordination, contextualization (from no background to dedicated background), representation (from most abstract to representation of details to the largest extent), illumination (from representation of light and shadow to the largest extent to no light and shadow), brightness (from different brightness in the largest amount to brightness and darkness only). Coding orientation demonstrates that, discourse is performed by specific social groups and in specific system situation, including technical, sensorial, abstract and natural coding orientations. Figure 1 is natural coding. Nearly every modality mark is of high value and belongs to high modality. It shows life-like scene. It seems that the coffee in readers' hand is smiling, and the foam that forms eyes and smile can be seen clearly.

3.2 Meaning construction of Cluster 2

Cluster 2 is composed of title and main body. The title expresses the theme of the advertisement, and it induces reader to read the main body. The main body explains the title and detailed states transitivity analysis of the content. It is shown in Table 2.

Gives, get (material process) and smile (behavioral process) dynamically express the manufacturer providing product to reader and the expected responses of readers, while relationship process statically describes the characteristics of product and the experience it brings.

Mood can be divided into indicative mood, interrogative mood and imperative mood. They represents four kinds of speech functions or speech act: statement (offer information), asking (seek information), offer (offer object and service) and order (seek object and service) (Jinying, 2014). The sentences in the copy are all indicative mood, and it aims to offer product information and expects readers to recognize related statement. In language, modality is the intermediate zone between affirmation and negation. It expresses the attitude of speaker to proposition or suggestion and can be divided into modality related to information exchange and modality related to product-service exchange. The purpose of this text is to offer information; therefore, it is related to modality. Except the high-value modality word can in the fourth sentence "Now that, s something you can smile about", there is almost no other modality. It demonstrates that, the manufacturer want to make information objective and real when describing products to readers, but it uses low modality for the proposition of the response of reader on product considering uncertainty and politeness strategy.

3.3 Meaning construction of Cluster 3

Cluster 3 is composed of image 2 and slogan. Slogan is the commercial terms repeatedly used during some period. It is usually fixed and mainly broadcasts the characteristics or concept of the product. "Good just got great" is the newly proposed slogan of Maxwell House and is the extension for the previous slogan "Good to the last drop".

As to representation meaning, image 2 is consistent with the product image on the website title of Maxwell House, and the highlighted brand name on the surface expresses both product and brand. As to interactive meaning, participator has no eye contact with reader but only offers information; participator has a full view demonstration with little space around, which expresses within the contactable range of reader; and the light overlook perspective expresses the reader is in a powerful position, and it seems that, they are standing by the share or desk full of products, which is often seen on advertisement. As to modality, it belongs to medium modality with blank background and deletion of detail depth, but the comparison of brightness and darkness become stronger, in order to highlight core content, that is, product and brand.

3.4 Composition meaning of multimodal discourse

Composition meaning of multimodal discourse is implemented by three synchronization principle: information value, salience and framing. First, information value refers to that, left and right, up and down, middle and edge structure of discourse delivers information value of known and new, ideal and real, center and auxiliary. This discourse mainly composes along horizontal axis and shows up and down structure. Cluster 1 on the top expresses ideal situation or generality of product. Below is Cluster 2 (title and main body) which is the practical detail information of product. Bottom is Cluster 3 which shows real and concrete product.

Salience refers to different degree of component attracting attention of looker and can be realized by placing in foreground or background, relative size and comparison of tonal value. In discourse, about half space is occupied by Cluster 1, therefore, it becomes the part with the strongest significance. Second, it highlights title "Here's to a world without bitterness" by size comparison (several times bigger than main body) and color comparison (white ground and blue words) in the discourse. Their purpose is to highlight the ideal situation and pleasure experience created by product.

Framing refers to with or without framing in multimodal discourse (can be realized by lines segmentation). These lines segment or connect the components in image. Although Cluster 1 is separated from the rest parts of discourse by the below framework line, the color of coffee cup is echoing with text in Cluster 2, bottle, tin and cup in Cluster 3 and color of slogan. The dominant tone is classical blue of Maxwell coffee, which integrates the whole multimodal discourse and achieves cohesion and coherence of the discourse.

3.5 Construction meaning of multimodal discourse

This discourse adopts distribution of window, that is, image is on the top and accounts for a large space, below is title and main body, bottom is product image, symbol and slogan, which is consistent with the skimming routine of from top to bottom. Cluster 1 with the strongest salience is the thematic element of the advertisement. The image shows high modality. The life-like scene and smile of personalized coffee to reader in close distance involves readers and shows equal relationship, in order to to achieve consistency and resonance on emotion with reader. Overall, this paper takes the taste of coffee as appeal point (without bitterness highlights the advantage and characteristics of product) and extends to life situation (Here's a world without bitterness). Smile and watching in figure 1 aims to affect the emotion of readers, and then establish emotion connection between brand and reader; it makes full use of emotional seeks of advertisement to effectively transfer the concept of creator to the target audience and offer them a new perspective, thus they generate new feeling and cognition on the product (Wenlian and Jianwei, 2014). As a result, it inspires reader's brand association on product, culture their positive emotion on product, stimulate their purchasing desire, finally, achieving interaction purpose. The analysis result of this discourse also represents the advantages and deficiency of words and image: visual impact of image is stronger, and its information transfer speed is faster than words, which is easy for reader to remember product information; language can detailed describe product information, further express the theme of advertisement, widen the imaginary space of reader. Therefore, the two patterns are both dispensable, and they strengthen each other and jointly coordinate to construct whole meaning.

4. Conclusions

We used system-functional linguistics and visual grammar to analyze the multimodal discourse of advertisement printed in English. The discourse analysis of advertisement printed in English mainly focused on the language analysis, but ignored the function of non-linguistic symbol resources on the discourse

meaning. Recently, the rising of multimodal discourse breaks through the limitation to a great extent. With system-functional linguistics of Hal Hday and visual grammar of Kress and Van Ixeuwen as theoretical framework, this study analyzed a piece of multimodal advertisement discourse, and found that, language and image forms joint forces by mutual strengthening and supplement by their own unique means, and jointly construct the whole meaning of discourse and obtain the best persuasion effect. But it is undeniable that the grammar analysis on the images has subjective, moreover, the interactive and complementary effect between the various modals are hard to confirm, therefore, to some degree, multimodal discourse analysis has some limitations, but as the more and more scholars begin to concern multimodal discourse, its analytic method and theory would be further improved.

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ANALYSIS OF THE INFLUENCE OF SKOPOS THEORY TO ENGLISH TRANSLATION OF FOOD PACKAGE

Yeyu Chen[1], Wenyuan Sun[1], Mengjia Liu[2], Yaling Liu[3], Qin Peng[4], Qiang Zhou[2], Rong Ma[*2]

[1]Foreign Language Department, Fujian Normal University Minnan Science and Technology Institute, Quanzhou, Fujian, 362332, China

*[2]Department of Chemistry and Life Sciences, Fujian Normal University Minnan Science and Technology Institute, Quanzhou, Fujian, 362332, China; *rmrmma@163.com*

[3]Foreign Language Institute, Tieling Normal College, Tieling, Liaoning, 112000, China

[4]College English Department, Xinxiang University, Xinxiang, Henan, 453000, China

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ABSTRACT

In recent years, more and more food manufacturers add English prescription on food package to enlarge the publicity for their products. But the translation quality is irregular and of bad readability, which severely violates the original intention of the publicity. This research introduced the Skopos theory of translation into the practice of English translation of food package. We reclassified the food packages according to their functions from the view of text type. We selected some cases and researched the translation strategy of different text types through analysis, qualitative analysis and under the guide of relevant theory of Skopos theory.In addition, the similarities and differences of consumer oriented "function" and the manufacturer oriented "intention" and the influence they bring to translation intention were compared. Finally, the enlightenment of translation Skopos theory to English translation of food package was concluded, as well as its development prospect in future application.

1. Introduction

Now, at the time of globalization, increasing Chinese products have gone abroad successfully. Wise manufactures have noticed the positive promotion of English translation to the popularization of the products, publicity of enterprise culture and establishing corporate image. So, foreign description mainly English has emerged on the food packages of many Chinese commodities. This research analyzed the English translation of food package. The food package of domestic food besides the food for export has gradually added English translation. The English translation provides channel for the foreign friends who don not know Chinese to acknowledge the food and enhance the publicity (Haidong, 2012; Yongchang, 2012). However, while we are cheering, we find that the translation of food package is irregular, the translation quality is low and the readability of English translation of many food packages is bad. They can not even satisfy the expression function of basic information, let alone publicize the products (Xiaofang, 2013). Thus it can be seen, it is of crucial practical significance to search for appropriate theory and method to research the English translation of food package and solve the practical problem. Translation Skopos Theory (called Skopos theory for short in the following content) is an important theory of German Functional School which advocates

that "intention decides the method". As translation is a behavior with intentions itself, the translation should be operated specifically according to the intention (Jihui, 2011). As Skopos theory has been introduced to China, there are many domestic scholars who have started trying to apply the theory in the translation research in specific filed. But, there are few research on applying Skopos theory in English translation of food package. In relevant literature of the research on applying Skopos theory in English translation of food package, researchers tried from different views, including translation principle, translation strategy, translation fault and pragmatic culture etc, and proposed their opinions (Zheng, 2011). Differing the previous researches that analyze the translation of food package in the perspectives of language structure, functional equivalence and translation fault etc, this paper tried to analyze real food package and reclassified the existing food package according to the functions applying the text type theory of Skopos theory frame, from the view of text type and under the guidance of Skopos theory. On that basis, we researched translation strategy of different text types applying relevant theory of Skopos theory. We applied the research results and the methods of the research in more similar fields and summarized the law, thus to improve the translation quality of food package.

2. Materials and methods

2.1 Materials

The ultimate purpose of our research on Skopos theory is to introduce the theory to the English translation practice of food package and make the theory play its role as guide. Before the formal sample analysis, it is necessary to classify the sample of the food package to make the research more convenient. The English translation cases of food packages this paper chose were real cases which comes from the market, network propaganda, presents given by friends and the precuts which have been exported. After the selection of materials, they were classified.

2.2 Sample classification and analytical method

We processed text classification to those English translation samples of food package using the text type theory of Skopos theory and processed qualitative analysis to the text with relevant theory and principle of Skopos theory (Skopos rule, coherence rule, fidelity rule and principle of functional and loyalty), including the analysis of function , intention, translation strategy and translation process etc.

Current food package contains several text types. They were classified into three categories according to Lace's text type theory which functions of different parts refer to: informative text, operative text and text of mixed types (Linqi and Xuan, 2011).

2.2.1 Informative text

Informative text of food package includes relevant information of the product, such as name, consistent, weight, usage, storage methods, expiration date, production address and executive sequence number etc. The function is to provide relevant information of the food to consumer, and the language is objective, acute, concise and logical.

According to Lace's text function theory, we treat the text about name, consistent, weight, usage, storage methods, expiration date, production address and executive sequence number etc as informative text. In food package, informative text plays definitely a dominant role.

The function is to provide absolute reality if the food, so the language is objective, precise, logical and without emotion, and the form is neat and orderly.

When translating, the translator should strive to reappears the content of original text precisely and comprehensively. However, the choice of text is restricted by the language and culture standard of target language.

2.2.2 Operative text

Operative text of food package includes advertisement language describing history origin, publicity of flow process, quality guarantee of product and etc which has great function of publicity and seducing. The language is usually with aesthetic feeling and alluring, which seems talking to the consumer face to face and which is more highly phatic. For example, the introduction of history origin, flow process and quality etc not only expresses the background of the food but also publicizes the product. The description and publicity was expected to enhance the confidence of consumer on the product and finally buy it. According to Lace's text type theory, the texts above belong to operative text.

To manufacturer, they never get bored advertising their product with the text on the food package, but whether the consumer would accept the advertisement from the manufacturer stay as a problem. So, as to operative text on food package, we should carefully analyze and compare if the consumer oriented function is consistent with the manufacturer oriented intention. There are two situations, one is consistent with intention and the other is inconsistent with intention.

2.2.3 Type of mixed text

The function of mixed type of food package can be simply summarized by a simplex text type. Sometimes, the text of mixed type has both the feature of informative and operative text, while express the information of food it has operative function. It has the function of advertising product and warning the consumer, such as trade mark, caution (sensitinogen etc,), reminder etc. Sometimes, it is neither informative text nor operative text but advocating ideas etc.

3. Results and discussions

3.1 Case analysis result of informative text

Case 1: Constituent description of handmade cakes produce by Kee Wah B akery

Original text:

成分：绿豆粉、砂糖、水、芥花子油、杏仁（木本坚果、）、花生酱（花生、砂糖、部分氢化植物油（含有大豆）、食盐）、椰丝、奶粉、杏仁霜（含有栗粉（含有麸质的谷类）、奶粉、杏仁（木本坚果）、大豆制品）、调味料及调味剂。

Translation:

Ingredients: Mung Bean Flour, Sugar, Water, Canola Oil, Almond (tree nut), Peanut Butter (peanut, sugar, partially hydrogenated vegetable oil (contains soybean), salt), Desiccated Coconut, Milk Powder, Almond Drink Mix (contains corn starch (cereals containing gluten), milk powder, almond (tree nut), soybean product), Flavor and Flavoring.

Function: inform the consumer with the food constituent.

Intention: express the information of food constituent.

Strategy: apply literal translation to the content and form, and domestication to culture.

Evaluation and analysis of the translation process:

In this case, the consumer oriented function is informing the food constituent, the manufacturer oriented intention is expressing information of food constituent, these two agree with each other and the translation intention is clear—translate the information of the food constituent (Yan, 2011).

According to the requirement of degree of accuracy of translation intention, translator should first comply with the fidelity rule of Skopos theory. The translation must be extremely loyalty to the original text to assure the full expression of the information of the original text. So, the translator applied literal translation in the translation of content and form, translated the information of the original text one by one, such as sugar, water etc. Meanwhile, according to the coherence rule of Skopos theory, the translation "should be coherent in the environment of communication

and the culture which accept it". Translator should consider the differences of two languages in culture cognition. During the literal translation, there were 5 groups of words which have been more subtly considered, Mung Bean Flour, Milk Powder, corn starch, Flavor and Flavoring.

In order to satisfy the demand of cultural cognition of consumer, according to coherence rule, the translation "should be coherent in the environment of communication and culture which accept it". Translator applied domesticating strategy in the aspect of culture cognition to make the translation more "authentic". The translator know well of that, to translate the food constituent more acute and objective the translator crossed the difference of cultural cognition between Chinese and English, chose appropriate words applying domesticating strategy and made the consumer clearer.

3.2 Case analysis result of operative text

3.2.1 Function conforms to intention

Case 2: Publicity of Shang Haojia Babao fruit drops

Original text:

"混合水果味的圆粒硬糖让您活力四射，流连忘返！"

Translation:

"A galaxy of sweet surprises, mixed with sweet and fruity flavors in this hard candy ball keeps you busy in your wake!"

Function: slogan with aesthetic feeling s to touch consumer and seduce them to buy.

Intention: advertises the fantastic function of the product and promote consumer to buy.

Strategy: domesticating strategy.

Evaluation and analysis of translation process:

The manufacturer oriented intention is almost consistent with the consumer oriented function, the purpose is clear: promote consumer to buy the product through advertising the fantastic function. The advertisement should be concise; the sentence pattern should have aesthetic feelings which cater the consumer (Danyun, 2011).

The translation intention decides the demand the translator should satisfy the consumer that is the requirement of coherence rule, the translation "should be coherent in the environment of communication and the culture which accepts it", conforms to the culture context of target language and be accepted by the readers of target language. And "the original text just provides some or whole information" for the target audience. The translator fully considered the appetite of target readers and consumer for advertisement and publicity and applied domesticating strategy when translating.

To satisfy the consumer psychology, the translation slightly transformed "流连忘返" to "A galaxy of sweet surprises" which means a lot of sweet surprise. Thought it is not equal with the original text and violets the fidelity rule of Skopos theory and it is not loyalty enough, it caters to the psychology of consumer who likes surprise. And the using of " A galaxy of " which means "a large number, as much as galaxy", to describe surprise can not only express the quantity but also shows the consumer a scene in which the galaxy is shining, gained better effect of publicity. What's more, the whole translation filled with aesthetic feelings and strong rhythm sensation, which conforms to the expectation of the consumer.

3.2.2 Function goes against intention

Case 3: Publicity of Prince of peace American ginseng instant tea

Original text:

野山花旗参是生长于自然森林中二十年以上的一种稀有的参类极品，其参味较甘，而且人参皂或含量最高，其性温和，不寒不燥。太子牌野山花旗参速溶茶是市面上唯一采用野山花旗参及美国威州花旗参为原料，

经严格挑选和品质鉴定，加以提炼，再配以上等葡萄糖精心加工而成，参味儿浓郁，外加防潮铝袋包装而成，充分保证野山花旗参之纯正品质及鲜味，是适合高度讲究并要求亲身体验皇室享受人士的最佳之选。

Translation:

This Prince of Peace Instant Wild American Ginseng Tea has been carefully selected, processed and packaged for your superior nutritional enjoyment from the rarest and highest value wild American Ginseng (panax quinquefolius) Root from Wisconsin. Naturally undisturbed grown in the forest, the American Wild Ginseng contains all the ginsenosides which some of them won't develop until the plant is over 20 years old.

Function: fulfill the publicity of product (respect the favor of consumer, delete the information which violets the demand of consumer).

Intention: make consumer accept all the information of the publicity about the product, enhance the favorable impression of the product in the mind of consumer thus promote consumer to purchase.

Evaluation and analysis of the translation process:

The manufacturer oriented intention aims to attract consumer to purchase through publicizing all the information of the product and enhancing the image in the mind of consumer to which the publicity can never be enough. But the consumer oriented function respects the favor of consumer and let consumer choose what they need and what they like. When the function and the intention is not in harmony, translator give consideration to both sides (Mingjuan and Jinghua, 2013).

According to the opinion of Skopos rule, "one of the most important factors which decide translation intention is the audience---for which the text is translated, they have their own cultural background, expectation for the translation and communication demands. Each translation points to particular audience" (Yan, 2013). When function and intention is in conflict, the feeling of target audience should be considered first, so the translation must consider the feeling of consumer first. It also meets the requirement of coherence rule which suggests "translation should be coherent in the environment of communication and the culture which accepts it". The mission of the translation is to publicize the product, to attract the consumer to accept the product and then buy it. The translator applied domesticating strategy which enabled the translation satisfies the consumer in many aspects such as language using and culture etc.

According to the principle of functional and loyalty in the frame of Skopos theory," translator is responsible for the translation sponsor morally" and "the purpose of target language text must be the same as the intention of the original author". Translator should accomplish the intended functions and respect the intention of the sponsor of the translation. The translator of this paper generalized and filtrated the information of the original text using information generalization strategy and focused on the expression of key functions. In order to highlight and emphasize the high quality and stimulate the consumer to buy, the translator put the part which was at the middle of the original text describes the quality of the product at the beginning of the translation, such as This Prince of Peace Instant Wild American Ginseng Tea has been carefully selected, processed and packaged. Putting the trademark at the beginning of the text can also enhance the memory and the publicity of the trademark.

3.3 Case analysis result of the type of mixed text

Case 4: Suggestions of the dietary standard of Prince of peace American ginseng

Original text:

每日百分率以日膳食 2000 路里为基础。

Translation:

Daily values are based on 2,000 calories.

Function: to remind the consumer with the dietary standard (it works only when exceeding a certain quantity)

Intention: show consumer the specific dietary standard thus they can control the consumption themselves.

Strategy: domesticating strategy.

Evaluation and analysis of the translation process:

In this translation, the consumer oriented and expected function is almost consistent with the manufacturer oriented intention. And the purpose is clear, show consumer the specific dietary standard thus they can control the consumption themselves (Xiaoling, 2009).

On the precondition of certain purpose and to express the information precisely, the translator, instead of translating one by one, resorted to the coherent rule of Skopos theory which suggests "translation fit in the culture of target language and its applicability should be accessed from the view of target language". So, to meet the consumer's demand better, the translation should apply domesticating strategy in the aspects of consumer language utilization and consumer psychology demand.

In language using, the translator chose passive sentence which conforms to the habit of food package language of western countries, and made the information more objective; deleted some redundant information of the original Chinese description which does not alter the understanding of the original text.

In the aspect of satisfying the psychology of consumer, the translation used only eight words to satisfy the consumer who is apt to suggestion information which is concise and with mass information, and saved the shopping time of consumer.

4. Conclusions

In conclusion, it can be seen, no matter informative text, operative text or the mixed text, the English translation of the food package is the same with the original Chinese text in text type, that is, parallel texts.

The main reason why target text and the original text are parallel texts is they have the same function. The specific function of informative text is to express information precisely with logical language. To both manufacturer and consumer the information expression of food package is more strictly required. So, the form and the content of the translation should be the same with the original text. The most common information is the constituent of the food package and date of manufacture etc (Zhuojia and Xuming, 2009). Operative text has to perform not only the function of information expression but also vocation function which attracts consumer to buy. So, the form is not highly required and is okay as long as it performs its function. Both target text and original text chose publicity discourse instead of the stylized form of informative text. The function of mixed text is complicated; some have both informative and advertising function, some have the caution function and even functions not relevant with the food. So, according to the demand of the function the translation text blends the form of informative text and mixed text.

The research of English translation of food package has theoretical and practical significance; the research of this direction will win more attention and find better method. And this research enlightens English translation of relevant fields and makes English translation be applied reasonably.

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Exploration of the Establishing of Practical Teaching Mode of the Applied Talents' Training Objective in Independent Colleges-Taking Food Science and Engineering Major for Example

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APPLICATION AND STUDY ON RAPID DETECTION OF BACILLUS CEREUS IN *food* BY COMPUTER VISION TECHNOLOGY

Shuying Liu

The Institute of Information Engineering of XianYang Normal University, Xianyang, Shaanxi ,712000, China
lsyxyxx@163.com

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ABSTRACT
With the increase of bromatotoxismus incidents induced by the foodborne pathogens, the study of detection methods of microbes in food both at home and abroad draws more and more attention. They are trying their best to avoid the defects in the traditional detection methods, so as to meet the need of ensuring the food quality and safety by detection, bacillus cereus is one of the most common foodborne pathogens which result in food poisoning, and it lives widely in the natural world in the form of spores. It can rapidly grow and propagate after infecting the food. It is likely to induce food poisoning once mistaken for food. Based on the computer vision technology and specific staining methods of bacillus cereus, this paper primarily discusses the rapid detection methods of bacillus cereus in food. This paper proposed some new ideas on the rapid detection of bacillus cereus in food, and extended the application of rapid detection system for microbes, thus to provide some theories and technology for supporting this system.

1. Introduction

Bacillus cereus is a kind of bacillus widely living the natural world whose gram stain is positive. Due to incorrect preservation method or preserved for too long, the bacillus begins to propagate in large quantities and produce toxins. Sometimes the contaminated food tastes sticky and not so god, there is no obvious phenomenon for spoilage. So that it is often mistaken for food and enjoys a high incidence of diseases, ranging from 60% to 100%. Bacillus is one of the common pathogens inducing the bacterial food poisoning.

As people pay attention to the food safety problems, there are more and more studies and researches on the rapid detection of pathogens in food both at home and abroad. Nowadays, there are a large number of studies on the detection methods for bacillus cereus, such as the ordinary detection methods and the rapid detection methods based on PCR and immunology technology. In China, we are based on National Standard GB/T 4789.14-2003 and the detection and Quarantine standard WS/T.82-1996. Besides, there are also some introductions and stipulations about the detection method for bacillus cereus, such as the SN 0176-92, MM_FS_CNJ_0340 (plate counting method) adopted in the export food detection and the SN/T 2206.2-2009 adopted in the cosmetics detection. However, for these methods are based on traditional ordinary detection, they cost a lot of time and energy (at least 3 or 5 days). It is likely to cause it hard to place supervision and control in processing, storage, transmission and sale. It is possible to lead to overstocking of products, which affect the timely shipping and sales. In this case, it may leave a heavy financial loss on the food enterprises, units and even the whole nation

(Sun Li, 2014, Zhang Zhihong, 2013, Zhang Yurong, 2013).

This paper mainly aims to realize the rapid detection of bacillus cereus in food by the computer vision technology (Xin, 2014; Ruiling, 2014; Yongguang, 2009). By analyzing the response from the bacillus cereus specific zymolyte, this paper built an image analysis system which gave a quantitative detection of bacillus cereus in food. With the help of the existing detection equipment in the lab, it optimized the characteristic parameter needed in image analysis. It also completed the data detection and analysis by the computer intelligence, and thus reached the goal of shortening the detection period and improving the detection quality (Zhengzhou, 2014; Zhibin, 2014, Guangyue, 2014). This kind of microbe rapid detection system, which is able to detect, handle and identify by its own, will take the place of the traditional manual detection method. It enjoys a lot of advantages, such as intelligent, time and labor-saving. Therefore, this study can not only provide important theory basis and academic value for this industry, but also leaves a broad prospect and considerable economic and social benefits.

2. Materials and methods

2.1 Experiment materials

Samples such as Rice noodle, rice and meat products which are available in the market. They turn bad by themselves or artificially for any purpose.

2.2 Experiment equipments

Microbe rapid inspector in type of WKJ-II (from the lab); portable pressure steam sterilizer in type of YXQ-280MD (from Jiaxin Zhongxin Medical Instrument Co., Ltd); Vertical Flow Clean Bench in type of ZHJH-C1214B (from Shanghai Zhicheng Analysis Instrument Manufacturing Co., Ltd); full temperature vertical shaking incubator in type of HZQF (from Harbin Donglian Electronic Technology Development Co., Ltd) and BG-highSPIN in type of TGL-16B.

2.3 Main culture bases and reagents:

Nutrient agar from Beijing AOBOX Biotechnology Co.Ltd. Accurately weigh 33.0 g, dissolve it with 1,000ml distillation water and then adjust the pH to 7.2±0.1. Keep it under autoclaving for 15 minutes at the temperature of 121^{o}C, and then set aside; the culture bases of liquid LB from the SIGMA Company. Accurately weigh 25.0 g, dissolve it with 1,000ml distillation water and then adjust the pH to 7.2±0.1. Keep it under autoclaving for 15 minutes at the temperature of 121^{o}C, and then set aside; MYP : 10.0 g peptone, 1.0 g beef extract,10.0g mannitol, 10.0g sodium chloride, 15.0 g agar, 1,000 mL distillation water, 13 mL 0.2% phenol red solution, 50 mL 50% yolk and polymyxin B. Add the first five ingredients in proper amount of spoiled water and then spoil. Add 13 mL 0.2% phenol red solution and have it diluted to 900 mL. Adjust the pH to 7.2±0.1 and keep it under autoclaving for 15 minutes at the temperature of 121^{o}C. When cooled to 50^{o}C, add 50% yolk and the sterilized polymyxin B (final concentration is 100 IU/ mL); X-GLUc dye liquor; 0.5% Peptone water solution and Methylene blue staining solution.

2.4 The experiment material disposal in the early stage

Weigh 25 g sample, and put it in the conical flask containing 55 mL Sodium phosphate buffer solution and then shake up. Put it into water bath at 65^{o}C and cross the heat treatment for 30min and then have it cooled to the room temperature. Draw 1mL sample into a 1.5 mL centrifuge tube with a sterile pipette, and then have it centrifuged by 110000 rpm for 10 min for spores enrichment, discard 0.85 mL supernatant, add 0.85 mL germination and then shake up to prepare spore suspension fluid. This suspension was subjected to a microwave treatment under conditions of high-grade 60s, and then has it rapidly cooled to room temperature.

Draw the 0.1 mL bacterial suspension and then put it in the centrifuge tube containing 0.9 mL short fermentation liquid. Next, have it

centrifuged by 150 rpm for about 2h. Concentrate the bacterial liquid with filter of which the aperture is 0.45 μm and the upward volume is 200 μL, then flush the centrifuge tube with 1 mL 0.5% peptone solution and filtrates them in the filter too. After that, flush the centrifuge with 1 mL 0.5% peptone solution again. Then, take down the filter and fill it with 2 mL gas through the bot. Absorb 5 μL bacterial liquid nearby the filter membrane, put them on the glass slide. When it is natural withered, add 6~8 μL staining fluid and cover the cover glass. Incubate for 10 to 20 minutes under the temperature of 37^{o}C and away from light, then flush then and process microscopic examination.

2.5. Rapid detection methods for *Bacillus cereus* that applies computer vision technology

In detection of *Bacillus cereus*, microwave was used to promote spore germination. Through short-term fermentation, bacillus cereus was stained into blue-green based on the staining function of X-gluc. Then computer vision recognition system was used for confirming and counting. The test process is shown in Figure 1.

Test separated *Bacillus cereus* by heating, and used microwave to promote germination of spore, and finally detect bacillus cereus after 2 h of short-term fermentation, filtering and condensing. This rapid detection method mainly used image identification technology after thallus coloring. Computer recognition program of bacillus cereus includes mid-value smoothing and denoising, RGD color space threshold segmentation, smoothing processing and edge extraction of mathematical morphology open + close calculation, eigenvalue extraction of perimeter, area, shape factor, rectangularity, elongation, BP neural network recognition, network model of 8-6-1 (Yurong et al., 2013).

3. Conclusion and Discussion

3.1 Detection results of *Bacillus Cereus* standard

Verification results of rapid detection of *Bacillus cereus* in food are shown in Table 1a and Table 1b. From the tables, we can see, there is still similar law with simulative sample between rapid detection and traditional cultivation. The relation of the two sample preparation methods was: B = kA, k = 3.0681; it is no big difference to the relationship coefficient of simulative model which was k = 3.0897. The operating error it brings can be artificially controlled.

From the detection results of applying traditional cultural method before and after sample preparation method, we can tell the average recovery rate of *Bacillus cereus* was 57.14%. The recovery rate of single sample was quite different when the recovery rate of single sample was compared. The probable reason is that *Bacillus cereus* does not always exists as the form of spore in some food. On appropriate condition, part of them presents as thallus which has weak resistance and lose activity when processed with high temperature and microwave etc.

From Table 1b, we can see, the average specific value of *Bacillus cereus* in food detected applying rapid detection and traditional national standard method respectively was 61.47% which was slightly bigger than the recovery rate of spore which was 57.14%. That is to say, the result of rapid detection is bigger than method of national standard which is probably because there is some infectious microbe or impurities with the same morphological characteristics and color characteristics as *Bacillus cereus* in the sample, caused erroneous judgment of detection system and thus brought deviation to the results. This deviation can be eliminated by applying prediction system such as regression model.

Besides, in theory, the detection limit of this rapid detection system is 20 CFU/g. But the sample demands complicated earlier stage processing especially the spore extraction and germinating stage affects greatly the results.

What's more, the sample we chose was of different kinds and forms, and the storing condition etc brought big difference to the sample room. So, from table 1, we can see the result variation of rapid detection system began to grow when the bacterium content of the sample is less than 50 CFU/g. Thus, the minimum detection limit of practical detection can be tentatively considered as 50 CFU/g.

The detection range of traditional plate cultivation which conforms to national standard is normally 15~150 CFU/g.

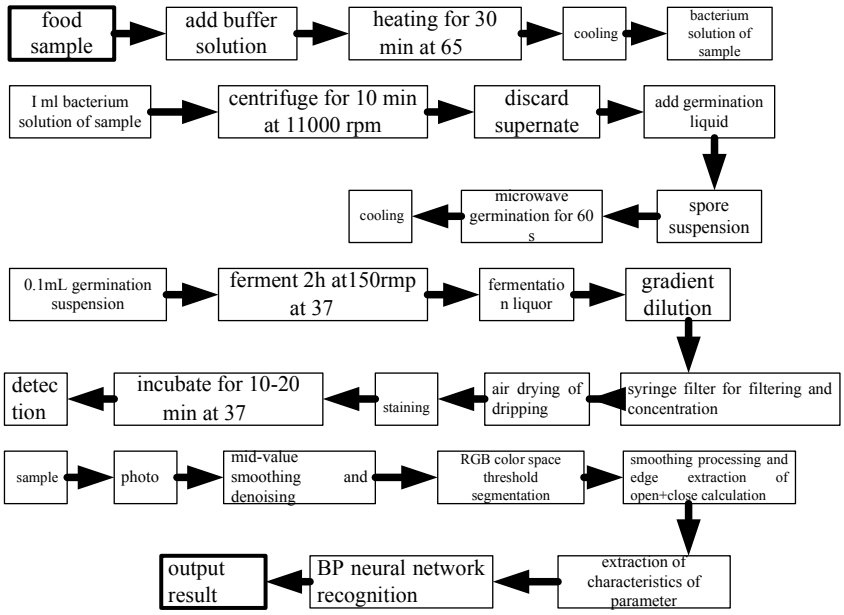

Figure 1. Examination process of *Bacillus cereus* by rapid detection method

Table 1a. Results of rapid detection and traditional detection (part)

Experimental sequence	Total quantity of thallus in the sample solution of traditional method A (CFU/ml)	Total quantity of thallus in the sample solution of rapid detection B (CFU/ml)	Total quantity of thallus in the sample solution after pretreatment of rapid detection C (CFU/ml)	Total quantity of thallus in the sample solution of rapid detection D (CFU/ml)
1	163500	510000	358000	349500
2	36500	114000	74000	75600
3	127500	398000	275000	268500
4	50	120	40	36
5	9	26	8	10

Table 1b. Results of rapid detection and traditional detection (part)

Experimental sequence	B/A	C/B	N/M	LogM	LogN	LogP
1	3.1193	0.6078	0.6283	6.2135	6.0117	5.9965
2	3.1233	0.6930	0.6628	5.5623	5.3837	5.4028
3	3.1216	0.6658	0.6739	6.1055	5.9341	5.9284
4	2.4000	0.3333	0.2304	2.6990	2.0615	2.1072
5	2.8889	0.3077	0.3556	1.9542	1.5051	1.4082

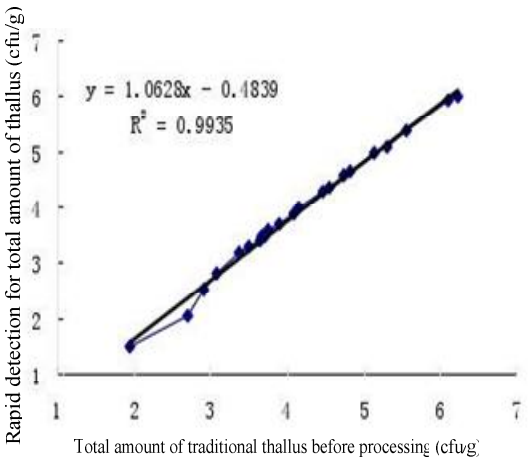

Figure 2a. Relationship of rapid detection and traditional detection for original sample

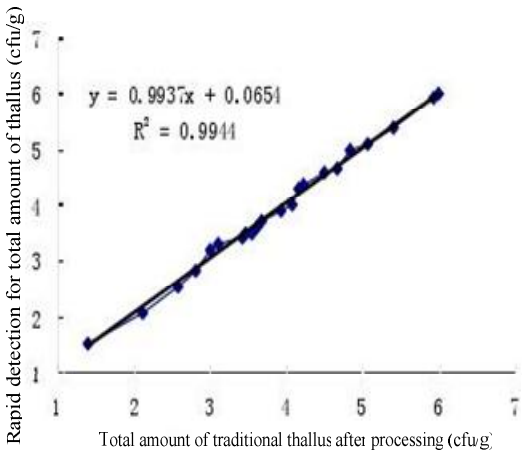

Figure 2b. Relationship of rapid detection and traditional detection

3.2 Correlations of rapid detection method and traditional detection method

Figures 2a and 2b are the results of correlation analysis of traditional detection method and rapid detection method before and after processing the commercially available samples. In can be seen from the figure that, the detection results of two methods were consistent, and showed good linear relationship. The correlation is 0.9935 and 0.9944, respectively.

In addition, the difference between them may be decreased after taking the logarithm and the value of R will be bigger since the practical detection numeral of this test is large. However, the error can be ignored when analyzing the correlation of these two methods. The above results and their analysis reconfirmed the rapid detection system was feasible for rapid detection of *Bacillus cereus*.

The difference is large since the samples selected have diverse categories and complex form. Therefore, there will be error in practical rapid detection. As shown in Figure 2, the second data has significant difference. As a result, establishing model for rapid detection of bacillus cereus in food still needed a lot of tests. The relationship of content of thallus and spore in food should be classified and discussed, since the existing state of *Bacillus cereus* in food is different.

4. Conclusion

At present, China has not limit the quantity of *Bacillus cereus* in food; however, state criteria points out that, it is dangerous when the content of bacillus cereus in food exceeds 104CFU/g(mL). Countries abroad limited the quantity of *Baciullus cereus* in food to 103~104 CFU/g(mL).

Through comparing the results of two detection methods for bacillus cereus in food, it was found that, the results of two methods showed linear relationship and had no statistical significance. Time of rapid detection method is only 5 h, and detection limit is 50cfu/g, which is superior than the traditional detection method. Through the accuracy analysis of rapid detection for *Bacillus cereus*, we can know that, food microbial rapid detection system adopted in this paper can not only accurately and rapidly detect the content of *Bacillus cereus* of food, but also has high specificity compared to the traditional detection method.

Bacillus cereus is the common conditioned pathogen in food. It is hard to satisfy the demand of real-time monitoring of food quality safety since the detection of bacillus cereus is generally using traditional plate count method with culture medium. In order to find out the rapid detection method, this paper introduced computer vision technology into the rapid detection of *Bacillus cereus*. Based on the color developing method based on the properties and specificity of *Bacillus cereus*, this paper initially discussed the rapid detection method for bacillus cereus in food. It not only proposes new idea for rapid detection of bacillus cereus in food and obtained certain achievements, but also widens the application range of microbial rapid detection, thus provide theoretical support and technical support for the improvement of microbial rapid detection system.

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CEREAL BARS – A HEALTHFUL CHOICE A REVIEW

Chetan Sharma[1]*, Amarjeet Kaur[1], Poonam Aggarwal[1], Baljit Singh[1]

[1]Department of Food Science and Technology,
*Punjab Agricultural UniversityLudhiana, *chn.pau@gmail.com*

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ABSTRACT
Recently, the consumption of fast-foods and snacks has increased, the desire for healthy and functional foods is also increasing at the same rate. In this sense the development of cereal bars formulation presents itself as an emerging force for this niche market. Epidemiological studies have repeatedly found that whole-grain (WG) cereal foods reduce the risk of several lifestyle-related diseases, though consistent clinical outcomes and mechanisms are elusive. Sales of nutrition bars, both as snacks and as meal replacements, jumped from $200 million in 1997 to nearly $1.7 billion in 2010. This review article presents the status, classification, possibly used ingredients and associated risks with the overwhelming market of cereal bars and helps to make a good choice. A wide variety of bars are available in the market but to chose the best suited one according to ones need will be catered by the current literature.

1. Introduction

American Heritage dictionary defines snack as "hurried or light meal" or "food eaten between meals." Traditionally, snack foods appeal to consumers on a number of levels such as taste, appearance, texture etc. Snack foods have always been a significant part of modern lifestyle, and they represent a distinct and constantly widening and changing group of food items. Today, designing of a snack food can be a complicated process to meet ever changing consumers' demands for taste and expectations, like "good for health," "rich source of protein," "offering a unique flavour" and the elusive search for something unique that also appeals to a wide variety of people. Globally, snacking is on the rise owing to an increasingly hectic lifestyle with more time spent at work. In the last ten years, changes in life-style and eating patterns have led to a gradual increase in demand for snack foods. The pattern of snacking in different countries can be affected by several factors such as the lifestyle in each area, the economic climate, rival foods and public receptiveness of current views on nutritional matters. Carefully designed nutritious snacks could help in decreasing under-nutrition problem and can provide an increased dietary intake of fibers, essential amino acids and other nutrients for developing countries.

Energetic and cereal bars have proliferated recently, and Americans spend a little over half of their food money on food prepared for eating without much – if any – further cooking (Katz, 1999). The consumption of cereal bars increased 11% globally in 2007, and it represents a market of about US $ 4 billion leading to an increase in the variety of cereal bars, labels, and different ingredients in order to attract consumers. Busy life styles and the increasing demand from consumers for meals and snacks that are quick sources of good nutrition have prompted the food industry to develop foods like nutrition bars that combine convenience and nutrition (Izzo and Niness,

2001). Accordingly, cereal bars emerged about a decade ago and represent an alternative food that is easy to consume while simultaneously possessing functional properties (Silva et al., 2009; Barbosa, 2007).

The increasing substitution of fresh foods rich in fibres, vitamins, and minerals for industrialized products, allied to a sedentary lifestyle favoured by changes in the work structure and technological advances, represents one of the major etiological factors in health related problems like obesity, arterial hypertension, dyslipidemias, cardiovascular diseases and metabolic syndrome etc. However, adequate nutrition allied to other modifications in the lifestyle contributes to the improved control of these associated risk factors preventing complications and increasing quality of life (Blonde, 2010; Donin et al*.,* 2010). The most recent research shows that the consumers are becoming more aware of the relationship between diet and diseases (Horn, 2006; Hollingsworth, 1997) and also gradual shift from animal-derived to plant-derived meals (Sloan, 1994). A number of phytochemicals present in plant foods have been shown to have a positive effect on health and disease prevention.

Cereal bars are made from a compressed mixture of cereals and dried fruit. Generally, the glucose syrup is the aggregator element of the bar ingredients providing rapid energy absorption. The idea behind cereal bars is that they provide a quick snack or breakfast for someone who is on the go. Assembling a full breakfast may be a challenge for someone with a tight schedule, and many people believe that breakfast is an important meal, so cereal bars fill the need. On other hand, since cereal is a complex carbohydrate, the starch provides slower energy liberation, which can be absorbed for a longer period of time. Cereal bars may be considered a good source of carbohydrate and may promote the recovery of energy following exercises when used as a food supplement or as "portable nutrition" (Brito, 2005). Therefore, the association between cereal bars and health food is a well-documented tendency in the food sector (Gutkoski, 2007).

Cereal bars are a popular and convenient food and, therefore, would be an ideal food format to deliver cereal or fruit-derived phenolic antioxidants and fibre. Cereal bars were introduced in the last decade as a wholesome alternative of comfit when consumers show more interest in health and diets. Since the consumption of cereals has expanded from the breakfast table to any time of the day, these products have become an excellent vehicle for the inclusion of functional ingredients in the consumers' diet. Therefore, a cereal bar could be considered as a practical choice for a quick meal due to its high nutritional value.

Since the consumption of cereals has expanded from the breakfast table to any time of the day, these products have become an excellent vehicle for the inclusion of functional ingredients in the consumers' diet. Cereals have been playing an increasingly vital role in modern lifestyles thanks to their varied uses including ready-to-eat products, instant products, cereal bars, and energy bars (Freitas and Moretti, 2006).

Classification

As cereal bars contained a wide range of ingredients and are prepared in lieu of nutritional demands of consumers, it becomes very difficult to classify them on some standard scale. From a nutritional point of view, the bars may be classified into four types: fibrous, energy, diet (light), and protein bars. Fibrous bars have a high fibers and glucose content with an energy value near 100 kcal per unit. The energy bars with 280 kcal provide easily absorbed energy because they contain less fiber and have a high caloric value. They are recommended for energy replacement following strenuous physical activity. Diet bars have only 65 calories are considered as sugar free. Finally, protein bars with approximately 17 g of protein per unit as well as a lower fat content makes it a good choice for protein-

loving and hard working peoples (Degaspari et al., 2008).

Ingredients commonly used in cereal bar manufacture

Nowadays, the consumption of fast-foods and snacks has increased, but the desire for healthy and functional foods increased at the same rate. In this sense the development of cereal bars formulation presents itself as an emerging force for this niche market. The nutritional value of cereal bars depends heavily on their ingredients used. The greatest difficulty in obtaining a good cereal bar is a combination of several ingredients with specific functionality such as vitamins, minerals, proteins, grains, fibers, thickening agents, sweeteners and flavourings, and turns them into a product with flavour, texture and decent appearance, while it tries to achieve goals specific nutrient (Lima, 2004). The development of a product is essentially a problem of optimization. In the search for the best formulation, the main objective is to determine the optimum levels of the components or key ingredients, which are the independent variables or factors and the dependent variable or response is the objective to be optimized (Dutcosky et al., 2006). The growing consumer demand for healthy, natural and convenient foods emphasis on to improve snack foods nutritional values via modifying their nutritive composition (Bhaskaran and Hardley, 2002; Gray et al., 2003).

The cereal bars have gained an acceptance in the consumers eyes as being ‘‘better for you’’ and good in nutritional terms, from the contribution of a amount of dietary fibre. The popularity of these products reflects nutritional guidelines recommending increased dietary fibre intake since low fibre consumption has been implicated as a risk factor in many diseases (Murphy, 2001). Insoluble fibre ingredients, such as bran, have traditionally been used in products such as cereal bars, breads, pasta and breakfast cereals, but the palatability of these has limited the level that can be incorporated into different systems. Soluble fibers ingredients are currently of greater interest in the formulation of ‘‘healthy’’ foods because they are more palatable.

The most commonly used ingredients in cereal bars are oat, wheat or rice, and soy with apparently no preservative. Generally, a source of syrup (agglutinant) is used to hold the dry ingredients. The most common choice of syrup or agglutinant is honey and/or sugar syrup. Ingredients like fruits, nuts, candy, and so forth may also be added to a cereal bar to enhance the flavour and taste. Some cereal bars are also dipped or coated with different coating materials like caramel or chocolate syrup. Malted or controlled germinated whole grain cereals could also be incorporated to increase the palatability and bioavailability of nutrients. During germination, enzymes are synthesized or activated to mobilize the storage compounds of the grain, leading to high enzyme activities and modification of nutrient bioavailability. Also, various bioactive compounds are formed in the metabolic processes. Larsson et al. (1996) reported an increased iron and zinc absorption from breakfast meals containing oat malt as a result of high phytase activity. Also, the amount of phenolic compounds, avenanthramides and phytosterols increase during germination. The total antioxidant activity of oat malt is comparable to that of butylated hydroxytoluene (BHT), a common food antioxidant (Makinen and Arendt, 2012). Buckwheat could also be used in the preparation of gluten-free products due to the presence of numerous nutraceutical compounds and rich in vitamins, especially those of B group. Moreover, buckwheat grains are a rich source of TDF (total dietary fibers), soluble dietary fiber (SDF), and could be applied in the prevention of obesity, celiac and diabetes.

Nutritional evaluation of the cereal bars

Consumers looking for a quick, convenient snack bar option are met with a host of possibilities in the market today, ranging from antioxidant-rich varieties, to cereal bars to help manage weight, to protein-rich options that would once have seemed to cater more to a

muscle-bound audience. Manufacturers are turning to super-fruits and added vitamins and minerals to stand out from the crowded cereal bar market.

As cereal bars are consumed as a meal replacement, it becomes important to seek out a cereal bar which provides nutrient at least equal to the regular meal. Many manufacturers make versions which are heavily sweetened, with a low fibers content and few vitamins or minerals. While these cereal bars may be tasty, they do not confer very much nutrition, and they may not provide the same benefits as a healthier version. There are some things to look for when buying cereal bars which can be used to select a product with the most nutritional value. The sugar content should be low while the fibers content should be high. High protein is another thing to look for, as are vitamins like C and A, with a cereal bar ideally having at least 25% of the daily value of two or more vitamins. Avoid cereal bars which are dipped in candy coatings, or cereal bars with ingredients like marshmallows, chocolate chunks, and other candy ingredients. Dried fruit and nuts are good ingredients to look for, as they can provide extra nutritional value. In addition to the key cereal ingredient, cereal bars usually have some sort of syrup which acts like a glue to pull the grain together so that it stays in a bar shape.

Epidemiological studies have pointed to diets rich in wholegrain (WG) cereal foods like cereal bars helps in reducing the risk of many diet-related diseases, including cardiovascular diseases, diabetes, obesity, the metabolic syndrome and some cancers, with similar results being found across many populations. The growing consumer demand for healthy, natural and convenient foods, attempts are being made to improve snack foods' nutritional values via modifying their nutritive composition. Snack bars are a popular and convenient food and, therefore, would be an ideal food format to deliver fruit-derived phenolic antioxidants and DF.

Consumers have easily accepted cereal bars because they are considered to be nutritionally balanced high-fiber snacks and also because they have an adequate balance between calories, fat, protein, vitamins, minerals, fibers and whole grains are beneficial to consumer health (Ryland et al., 2010).

Different methods for the preparation of cereal bars

Generally, there are many methods for the preparation of cereal bars. Every bar has its own method of preparation. However, broadly the methods of preparation can be divided into two classes: (i) Hot/Oven process and (ii) Cold process.

In hot or oven process of bar making all the dried ingredients are mixed with agglutinant and baked whereas in cold process method all the ingredients are mixed and then moulding is done to shape them or stored in cool temperature/refrigerated as shown in Figure 1.

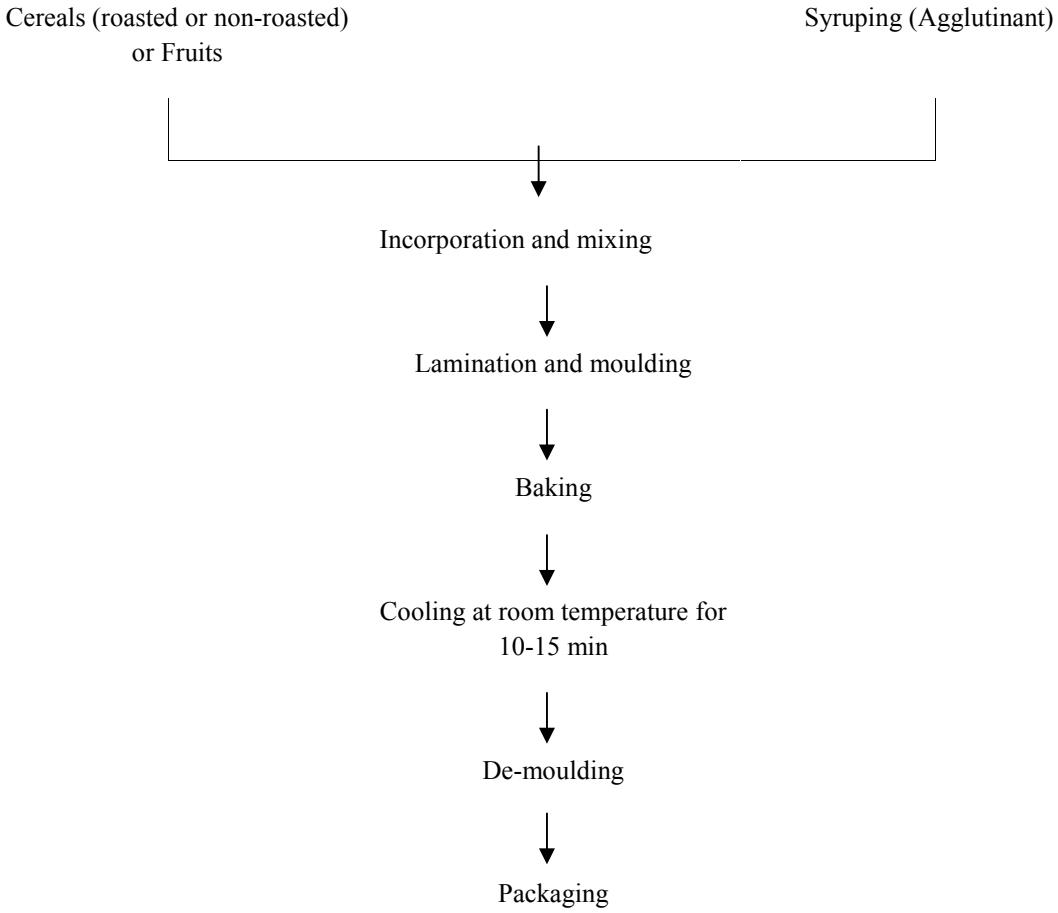
Associated risks and labeling

Approximately 25% of the United States population believes that they have an allergic reaction to foods. However, the actual incidence confirmed by history and challenges suggests a prevalence rate closer to 2-8% in young infants and less than 2% in adults. The number of food allergy and intolerance sufferers in the U.S. ranges from 6-11 million people, based on estimates from the FDA and the Food Allergy & Anaphylaxis Network (FAAN). The most common food allergies in the United States are milk, egg, peanut, soy, wheat, tree nuts, fish and shellfish. As cereal bars contain a wide variety of ingredients it becomes necessary to label all the ingredients and possible allergens clearly. For example, in year 2004, gluten-free labeling was virtually non-existent. However, in coming years, all of the newly launched snack bars are completely labeling their ingredients and even ingredients which are used in less than 1 % level.

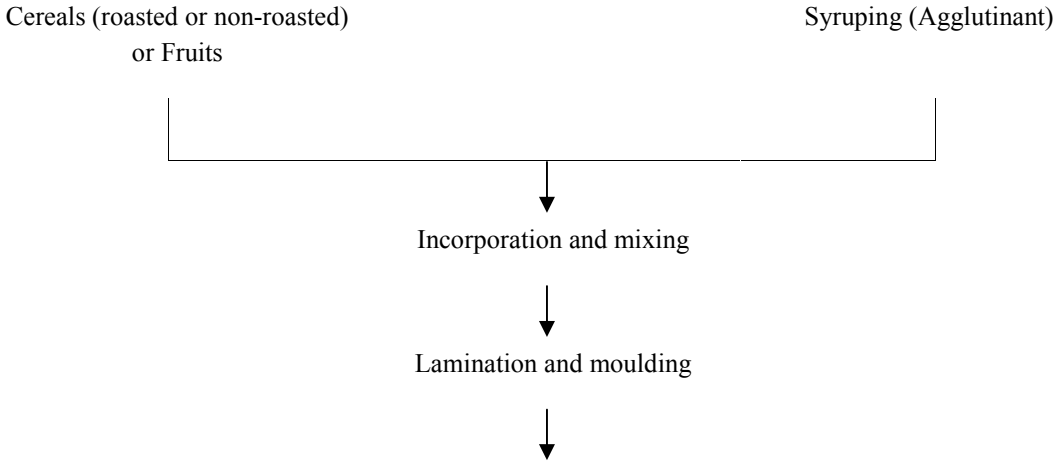
This presents an opportunity for manufacturers of rice, oat, pea and other allergen-free flours to formulate existing bars with these allergen-free flours. Presently, the majority of bars have some form of wheat

present in the formulation, albeit used minimally as functional ingredients. It may be possible to replace wheat and soy flours to enable more manufacturers to provide allergen-free, gluten-free food products with more consumer demands.

Hot-process:



Cold process:



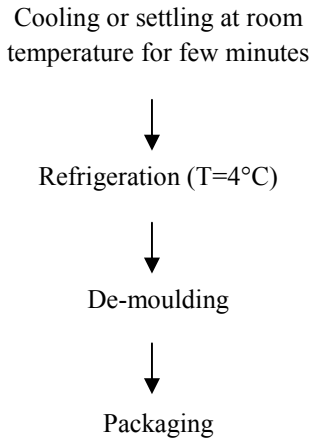


Figure 1. Flow diagram of bar preparation

Other important concerns in the selection of bars are their caloric content, hydrogenated fats and sodium content. Some types of bars available in the market contained the equivalent of almost 4 teaspoons of sugar and higher levels of fats too. Some bars even contain more than 30 % of sugar. Some days back news was printed in leading newspaper (Daily Mail), where it was shown that bars even contain more sugar (18 g) than that of a 150 ml cola can (15.9 g) contained. The presence of fats or hydrogenated fats which enhances the risk of cholesterol in the blood plasma poses another risk in consumers mind. Some cereal bars aimed at children were found high in saturated fats. Fat content should be as low as possible. Lastly, low levels of sodium also reduce the associated problems with blood pressure.

In context of labeling and packaging, some types of "traffic light coloured food labeling" is being promote and popularized as a clear and more informative indication of healthier products and possible ingredients. Food companies are increasingly including nutrition information not just in the Nutrition Facts panel but also on the front-of-package. This front-of-package information can influence people's choice about which foods to buy. UK Food Standards Agency's voluntary traffic light system is an example of a different type of front-of-package labeling. The symbols provide both specific nutrition information and gradations about positive or negative levels of fats, saturated fats, salt and sugars. A red light indicates a high level, amber light indicates a medium level and green light indicates a low level. Traffic light symbols provide consumers with a lot of information that can be gathered in a glance. "Guiding stars" is another system, developed by Hannaford Supermarkets includes stars as an index of shelf labeling. The more stars, the healthier the food under Guiding stars criteria.

Current trends and future prospects

This sector is also considered very poor in terms of availability of raw materials. Besides cereal products, there are only few new options being offered to consumers. In fact, as *Packaged Facts* noted in a report earlier in 2012, cereal and granola bars have met with competition from not only nutrition and energy bars, but also with an array of other snacks perceived as healthier—including cheese, yogurt, trail mixes, extruded snacks etc.

Since 2007, the cereal bar segment has become more narrowly focused on health and weight management. Products introduced in the past few years have emphasized functional health benefits, all-natural and organic ingredients, taste, flavour and variety. An especially popular attribute is added fibers in all these cereal bars.

Many new bars in the market feature lifestyle or wellness benefits, such as organic ingredients, antioxidants, high fibers and probiotics. Taking this a step further, cereal bar manufacturers can follow the lead of product developments in the functional food and drink markets and incorporate additives that have risen in prominence in recent years, like glucosamines, ergosterols, coenzyme Q10 and omega-3 fatty acids. Resveratrol's antioxidant benefits have been widely touted in wines, but one recent bar introduction brought that consumer recognition into a new segment. Antioxidant enrichment is the primary selling point of cereal bars. The bars promise antioxidant vitamins C and E through a mix of almonds, blueberries, cranberries and peanuts.

2. Conclusions

The market is flooded with granola and cereal bars of high protein and high energy values but there are a wide scope for further nutrient enrichment of bars through addition of essential nutrients rich ingredients. This will not only meet the growing consumer demands but will also cater the several existing nutritional deficiencies in the developing nations. Further, the use of seasonal fruits in the form of pulp and high in nutrition can also make them cost effective. Despite a noticeable increase in consumption, it is to believe that the research on market expectations and consumption is still incomplete and need further targeted study.

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THE INFLUENCE OF ROASTING PROCESS ON THE PHYSICO-CHEMICAL CHARACTERISTICS OF ARABICA INDIA COFFEE BEANS

Bianca Cimpan*

*Technical University Cluj Napoca, North Universitary Center of Baia Mare, Department of Chemistry and Biology, Victor Babes Str., 76, 430083, Baia Mare, Romania, * bianca_cimpan@yahoo.com*

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ABSTRACT
This work aims to study the influence of roasting process on the physical-chemical characteristics of Arabica India coffee. Coffee is one of the most marketed products, consumed daily by most people and for this reason we have chosen to study the properties of coffee and its behaviour during roasting. Data about the characteristics of green coffee are presented in the present article. The following parameters were measured: content of polyphenols, mass loss during roasting and drying, the solubility of the mineral elements and of the polyphenols and the content of caffeine.

1. Introduction

Within a few years the Dutch colonies had become the main suppliers of coffee to Europe. The Dutch were growing coffee at Malabar in India, and in 1699 took some to Batavia in Java, in what is now Indonesia. Today Indonesia is the fourth largest exporter of coffee in the world (Ranken, 1997).

For North Americans, the world's largest consumers, Seattle is the new spiritual home of coffee. The wettest major city in the USA gave birth in the 1970s to a café or 'Latte' culture which swept the USA and has dramatically improved the general quality of the coffee Americans drink. Today, any public place in the USA will have one or more coffee carts, serving a variety of coffees, drinks and snacks (Ranken, 1997).

This new found 'coffee culture' has started to spread to the rest of the world. To those countries with great coffee traditions of their own, such as Italy, Germany, and Scandinavian countries, added new converts to the pleasures of good coffee. Today it is possible to find good coffee in every major city of the world, from London to Sydney to Tokyo. Tomorrow the world will drink more and more importantly, better coffee (Ranken, 1997).

The importance of coffee in the world economy cannot be overstated. It is one of the most valuable primary products in world trade, in many years second in value only to oil as a source of foreign exchange to developing countries. Its cultivation, processing, trading, transportation and marketing provide employment for millions of people worldwide. Coffee is crucial to the economies and politics of many developing countries; for many of the world's Least Developed Countries, exports of coffee account for a substantial part of their foreign exchange earnings in some cases over 80% (www.harvestsoftheworld.com). Fruits (cherries) coffee tree reach full maturity after eight or nine months after the appearance of the flowers, or even after 11 months, such as the Robust species. They have first a greenish tinge, which then changes color in yellow and ripening as they become saturated red color

changing again in purple shiny when they reach full maturity. Harvest time varies with condition pedo-climatic and geographical region where coffee trees are grown. (Mandruta, 2008). Ideally beans should be a uniform blue, bluish grey or greyish green. In addition to colour, the shape of the beans is also critically assessed. Large well-rounded beans are considered desirable, while poorly shaped or 'ragged' beans may be thin, pointed or even boat shaped and are poorly regarded.

During roasting, significant chemical changes occur which are responsible for the development of the desirable flavour. Most important is the formation of an extremely complex mixture of volatile aroma constituents. While they constitute only about 0.04% of the weight of the roasted bean, they are responsible for most aspects of flavour and aroma **(**Ranken, 1997).

Caffeine is chemically stable under roasting conditions, but a small proportion may sublime. After roasting a sample under carefully controlled and standardized conditions, the beans should ideally be a shiny uniform dark brown (Ranken, 1997).

The aim of this study is to test the influence of roasting process on the physico-chemical characteristics of Arabica India coffee.

2. Materials and methods

The green coffee was dryied in oven at 100^{o}C for 90 minutes and roasted on a hob at 100 ^{o}C and 200 ^{o}C for 40 minutes.

2.1. Humidity of green coffee

A coffee sample of 4 g is kept 48 hours at 105±2^{0}C until the mass is constant.

$$\text{Humidity}(\%) = \frac{m_1 - m_2}{m_2} 100 \qquad (1)$$

where: m_1 – coffee sample weight subjected to drying out (g), m_2 – coffee sample weight after drying (g).

2.2. Density of green coffee

The density was determined with the Eq. 2. The mass of 100 coffee beans was measured and the volume of 100 coffee beans was determined by using the pycnometer (Mihaly-Cozmuta, 2011).

$$\text{Density}\ (g/cm^3) = \frac{m}{V} \qquad (2)$$

where: m – mass of 100 coffee beans (g), V – 100 coffee beans volume (cm^3)

2.3. Soluble fraction of green coffee

A volume of 200 mL hot water was added over 2 g of ground coffee and the mixture was stirred for 1h. Then the mixture was filtered and the supernatant was dilluted in a 500 mL flask with distilled water. Then, a volume of 50 mL of the dilluted extract was evaporated (105±2^{o}C). The mass of the solid residue was measured. The soluble fraction was determined with the Eq. 3: [Cozmuța, 2011]

$$\text{Soluble fraction}\ (\%) = \frac{(m_1 - m_2)x10}{m_3} 100 \qquad (3)$$

where: m_1 – mass of the dish with the solid residue (g), m_2 – mass of the empty dish (g), m_3 – mass of the analyzed coffee sample (g), 10 – correlation factor of sample volume.

2.4. pH of green coffee

A volume of 50 mL hot water was added over 3 g of ground coffee and keep at room temperature for about 1 h. The pH was measured with the Inolab pH-meter.

2.5. Total acidity of green coffee

A volume of 75 mL ethanol (80%) is mixed for 16 hours with 10 g ground coffee. The mixture was filtered and a portion of 5 mL liquid phase was titrated with NaOH 0,1N solution using the phenolphthalein as indicator, until pink color is weak persistence for at least 1 min (Mihaly-Cozmuta, 2011). The acidity was determined with the Eq. 4:

$$A\ (^{o}T) = 2VF \qquad (4)$$

where: A - acidity, m – mass of the sample (g), V – the volume of the solution NaOH 0,1N used for the titration (mL), F – solution factor of NaOH (F=1).

2.5. Fat content of green coffee

The fat content was determined by the extraction method, using the VELP extractor. An amount of 5 g ground coffee was weighed in a cotton flask. A volume of 60 mL ethanol was used as solvent. The procedure consists in 3 stages: immersion (120 min); washing (20 mi) and recovery of the solvent (1 min). The extraction temperature was 210 ºC. The capsule is let for dry in nice, at room temperature and then it is weight. The fat content was determined according to the Eq. 5:

$$\text{Fat content}\,(\%) = \frac{(m_2 - m_3)}{m_1} 100 \qquad (5)$$

where: m_1 – mass of the coffee sample (g), m_2 – mass of the capsule of extraction and fat (g), m_3 – mass of empty capsule (g).

2.6. The loss of mass on drying/heating of green coffee

Samples of 20 coffee beans were weight and then subjected to the drying (in the oven at 100ºC) and roasting processes (on iron at 100ºC and 200ºC). The sample was weight until the constant mass. The mass loss was determined according to the Eq. 6:

$$\text{Mass loss}\,(\%) = \frac{(m_i - m_f)}{m_i} 100 \qquad (6)$$

where: m_i – mass of the coffee sample subjected to drying out and roasting (g), m_f – mass of the coffee sample after drying and roasting (g).

2.7. Polyphenols content

Determination of the polyphenols content in the green coffee was performed according to the Folin-Ciocalteu method by following the next steps:

2.7.1. Preparation of the calibration curve A standard solution (2 mg/mL) of gallic acid was prepared by dissolving 0.01 g galic acid in 50 ml distilled water. The standard solution was used to prepare the solutions of 1.5 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.2 mg/mL and 0.1 mg/mL concentrations. The absorbance at 765 nm of the prepared solutions was measured using the Perkin Elmer Lambda 35 spectrometer. The dependence of the absorbance as a function of the concentration was reprezented (calibration curve). The concentrations and absorbance values and the calibration curve are presented in Figure 1.

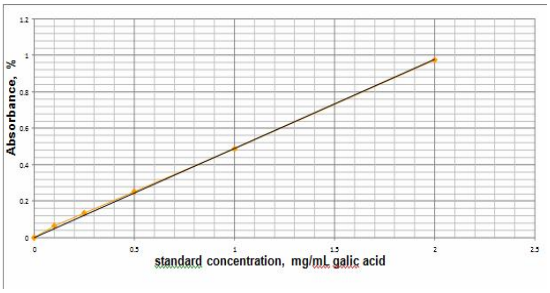


Figure 1. Absorbance against of gallic acid concentration

2.7.2. Extraction of the polyphenols

*A*n amount of 0.5 g ground and fried coffee was homogenized with 10 ml methanol solution (70 %) and was heated in the water bath at 70 ºC for 10 min. Then, the sample was centrifuged. Into a 25 ml volumetric flask containing 9 ml of distilled water, a volume of 1 mL filtrate, 1mL standard solution of gallic acid and 1mL Folin-Ciocalteu were added and the mixture was homogenized. After 5 min, a volume of 10 mL Na_2CO_3 7% solution was added and the thus prepared solution was immediately diluted with distilled water up to the sign. The solution was kept at room temperature, for 90 min and, then, the absorbance at 765 nm was measured. The concentration of polyphenols was determined from the calibration curve and was expressed in mg/g of gallic acid.

2.8. Solubilizing of mineral elements and polyphenols from roasted coffee

2.8.1.Solubilizing mineral elements

A weighted sample of green coffee was calcined at 540ºC, gradually raising the temperature. The resulting ash was

homogenized with 20 ml of HNO_3 0,1N solution. The mixture was filtered into a volumetric flask of 50 ml and was diluted up to sign with distilled water. The final solution was used to determine the concentration of mineral elements using the atomic absorption spectrometer Perkin Elmer 850.

A sample of the coffee was also roasted at 200ºC for 5 min and then was grinded. An amount of 3 g ground coffee was diluted with 75 mL hot water and was kept different periods (10, 20, 30, 40, 50 min) to infuse. The resulted solutions were filtered and the concentration of mineral elements was spectrophotometrically determined.

2.8.2.Solubilizing polyphenols

In a 25 ml volumetric flask containing 9 ml of distilled water, 1 mL from the solutions sampled at different moments of infusion was added together with 1 mL standard solution of gallic acid, 1 mL Folin-Ciocalteu and the mixture was homogenized. After 5 min, a volume of 10 mL Na_2CO_3 7% was added and the thus prepared solution was immediately diluted with distilled water up to the sign. The solution was kept at room temperature, for 90 min and, then, the absorbance at 765 nm was measured.

2.9. Determination of the content of caffeine in coffee

2.9.1.Caffeine extraction

Coffee beans are ground and grinded through 0.2 mm sieve to get a uniform texture. An accurately weighed amount of sieved coffee (approximately 10 mg) was dissolved in 5 mL of distilled water. The solution was magnetically stirred for 1h and heated gently to remove caffeine easily from the solution. In addition, the solution was filtered by a glass filter, mixed with dichloromethane (5:5) for the extraction of the caffeine from the coffee beans (extraction time 10 minutes). This procedure is repeated for 2 times with 5 mL dichloromethane and the extract was collected in a glass. The absorbance was measured using the UV-VIS Perkin Elmer Lambda 35 spectrometer at 274.7 nm.

2.9.2. Calibration curve

Samples of 0.0001 g, 0.0005g, 0.001 g, 0.004 g and 0.008 g caffeine dissolved in 10 mL of dichloromethane, a significant measuring at the same wavelength used for the investigated sample. Knowing the absorbance of samples of caffeine, the calibration curve is drawn and on the basis thereof shall determine the concentration of caffeine in the samples to be analyzed. The concentrations and absorbance values and the calibration curve are presented in Figure 2.

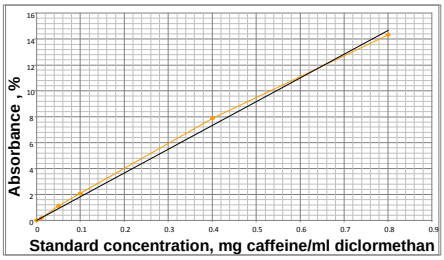


Figure 2. Calibration curve of the caffeine

3. Results and discussions

3.1. Physical parameters of the green coffee

In Table 1 are presented the obtained values of the investigated parameters.

Table 1. The values of investigated parameters

Parameter	Experimental results	Values in literature
Humidity, %	11.03	11.15
Density, g/cm^3	1.10	
Soluble fraction, %	26.18	30.80
pH	6.06	6.12
Total acidity, ^{o}T	2.75	5.77
Fat content, %	12.17	13.40

The loss of mass of green coffee during roasting is presented in Figure 3 while in Figure 4 is displayed the macroscopic aspect of

coffee beans during roasting at 100°C and 200°C, respectivelly.

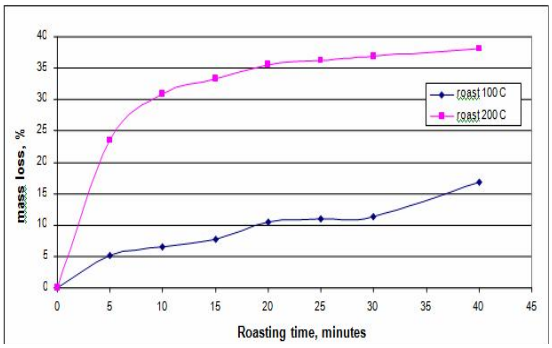


Figure 3. The loss of mass obtained by roasting

Figure 4. Macroscopic view of the coffee samples during roasting at 100^0C and 200^0C

The coffee roasted at 100^0C is subject to lose a share of 7.7 % in the first 15 minutes, after which the reached 16.9% loss in 40 minutes. In comparison with the roasting at 100^0C, the coffee roasted at 200°C have lost 23.4% in the first 5 minutes, and at the end of the process the loss reaches 38%. This loss is due to the heat treatment much more intensely. During roasting, the coffee loses a large amount of water and volatile substances. The first snap was made in 5 minutes at temperature 200^0C.

3.2. Chemical parameters of the green coffee

3.2.1. Polyphenols content in roasted coffee

In Figure 5 is presented the evolution of the polyphenols content in coffee beans during roasting process.

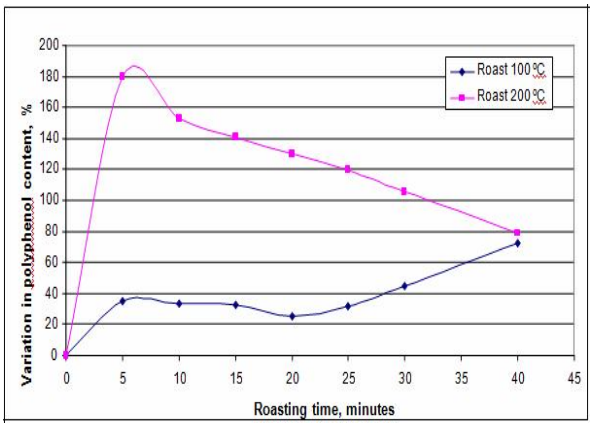


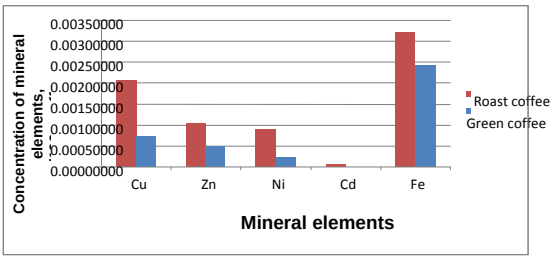
Figure 5. The variation in polyphenols content of coffee beans during roasting

The initial concentration of polyphenols during roasting at 100^0C increases within the first 5 min, due to the evaporation of water and volatile substances from the coffee beans. Then, the content remains constant within the next 20 min, after which increases up to 13118.118 mg/100 g coffee in the last 15 min, due to the acceleration of the rate of processes in which the polyphenols are generated.

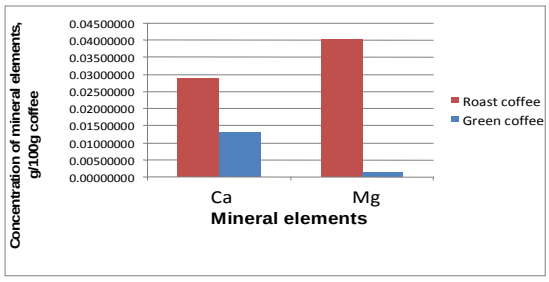
In the case of coffee roasted at 200^0C, the amount of polyphenols is reaching a maximum after 5 min. The increase of the content of polyphenols is due to the evaporation of water and volatile substances and also to polyphenols generating processes. In the next 35 min, the content of polyphenols significantly decreases up to 13588.153 mg/100 g coffee, because of the oxidative processes that are more accentuated. In both cases of roasting, the content of polyphenols after 40 min is higher than the content of the polyphenols in the green coffee. It further notes the values of content of polyphenols during roasting at 200^0C are higher than the values obtained after roasting at 100^0C, due to more intense thermal regime.

3.2.2. Solubilizing mineral elements and polyphenols from coffee.

Figure 6 shows the content of mineral elements in green and roasted coffee beans, respectivelly.



a



b

Figure 6. Content of minerals in the coffee Cu, Zn, Ni, Cd and Fe (a) and Ca, Mg (b)

Apparently, they seem to be in a larger amount in the roasted coffee than in the green coffee, due to the evaporation of water thermal processing. The contents of Ca and Mg in coffee are higher than those of Cu, Zn, Ni, Fe, Cd.

In Figure 7 are presented the values of the mineral content in the coffee solution.

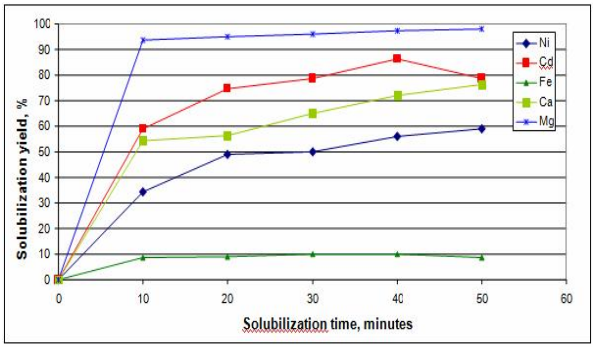


Figure 7. Mineral content in the coffee solution

Yield of solubilization of Mg is nearly 100%, and in the opposite corner is Pb, Zn, Cu and Cr which are not soluble. The yield of solubilization of Fe has a maximum of 10% for 30 minutes. The leaching of Ca, Ni and Ca is gradually during 50 minutes.

In Figure 8 is presented the content of the polyphenols in the coffee solution.

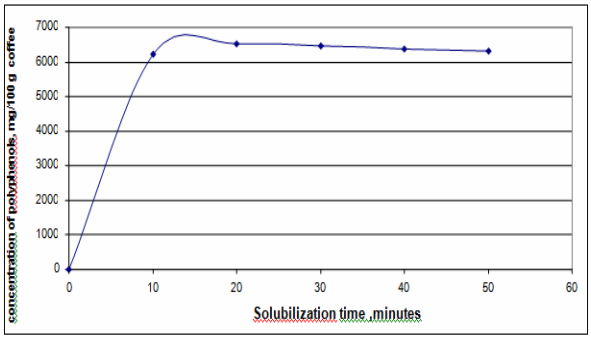


Figure 8. The content of polyphenols in the coffee solution

The largest concentration of polyphenols in coffee solution is in the first 20 minutes. After this time, the concentration of polyphenols decreased slightly due to oxidation processes. Polyphenols solubilization of the Yield is low. Only 8-8.5% of the amount of polyphenols in roasted coffee is transfered in coffee solution.

3.3.3. Caffeine content

In Figure 9 is presented the concentration of caffeine in the coffee roasted at 200^{0}C.

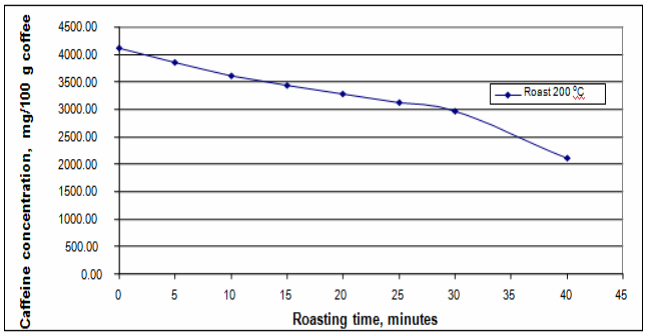


Figure 9. The concentration of caffeine in the coffee roasted at 200^{0}C

Figure 10 presents the evolution of caffeine level during coffee beans roasting at 200^{0}C. The concentration of the caffeine in the roasted coffee gradually decreases in the first 30 min at 4107.84 mg/100 g coffee until 2970.00 mg caffeine/100 g coffee, then within the next 10 min to 2108.91 mg caffeine/100 g coffee.

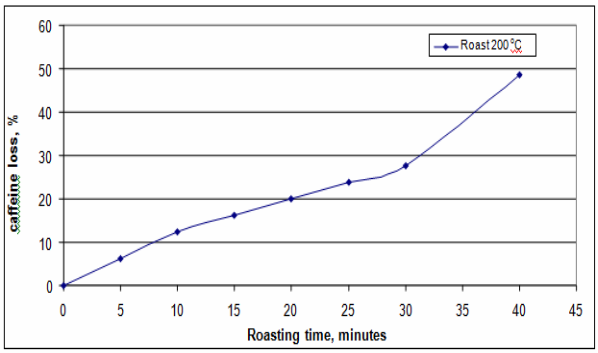


Figure 10. Loss of caffeine content in the coffee roasted at 200^0C

This decrease is due to the heat treatment and due to the long roasting time. After 40 min of roasting, the coffee loses about 50% of the amount of caffeine present in green coffee.

4. Conclusions

This work aims to study the influence of roasting process on the physical-chemical characteristics of Arabica India coffee. The roasted coffee is subject to lose a greater amount of its mass from drying, due to more intensive heat treatment. During the drying process, the formation of polyphenols is present, while during the roasting, this process occurs much more intense. Temperature range between 200 and 218^0C means "tip flavor" for roasting, therefore for solubilizing the mineral elements and polyphenols in coffee solution I have chosen a temperature of roasting of coffee beans 200^0C for 5 minutes. If a person wants to consume a coffee with a high concentration of polyphenols (antioxidants), it must have a drink in the first 20 min after its preparation. Also, to get a cup of coffee with an appreciable content of caffeine, it is recommended that all the while roasting at 200^0C.

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A SURVEY ON MILK ADULTERATION AT RETAIL OUTLETS OF ISLAMABAD, PAKISTAN

Tariq Aziz[1], **Haider Khan**[1*]

[1]*Dairy and Meat Technology Program, Animal Sciences Institute, National Agricultural research Centre, Islamabad, Pakistan; *haiderkhan_1@hotmail.com*

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ABSTRACT

Milk, being highly perishable, is the most frequently inspected food commodity. In raw form it is best preserved by chilling and where this is not possible adulteration is likely to happen and may prevail throughout supply chain. It is generally blamed by corporate sector that policy and practice of raw (unprocessed) loose milk retailing is both cause and effect of milk adulteration. In order to assess the severity of the problem and truth of allegation a study was planned in which samples from the milk retail market of Pakistan capital, (Islamabad) were taken on weekly basis in the year 2011 and were tested for their milk composition and presence of chemical adulterants. It was found that summer/hot season is the peak time of practice of chemical adulteration. This practice started appearing in the month of May and then it suddenly shot up and remained more or less consistently high in June and August and afterward started declining till it touched rock bottom in winter months. As regards incidence of various chemical adulterants the most prevalent was carbonate (27%), which was followed, in order of decreasing incidence, by hydrogen peroxide (7%) and starch (5%). The compositional quality of milk was also very poor. Extraneous water as an adulterant was found almost in all samples; the incidence was 99 % and 88 % with respect to buffalo and cow milk standards of Pakistan respectively, the respective average of added/extraneous water was 36 and 25 percent. It is concluded that adulteration, particularly the extraneous water, apart from added carbonate, is serious problem of loose / retail milk market and strict and strategic measures are required to safeguard consumer rights and their health.

1. Introduction

In many developing countries, particularly in South-East Asia the economies are largely agrarian with subsistence livestock production / farming as an integral part. The corporate sector is not yet involved significantly in agricultural production. In Pakistan, as is the case with India, Nepal, Bangladesh and many African countries such as Kenya, Tanzania etc, *bulk* of milk is produced in remote areas and it takes usually long time to reach urban markets where milk is in high demand. Being highly perishable food, its handling and preservation warrants very stringent measures to avoid losses or devaluation of milk and the best way to avoid bacterial spoilage is by keeping it chilled until consumed or processed. But due to poor milk collection system prevailing in Pakistan which largely lacks chilling facilities and transport infrastructure, shelf-life and other quality parameters of milk are severely deteriorated before its disposal to far away urban markets and processors. This is especially true for the bulk of milk (90 to 95 %) handled by unorganized and informal sector involved in milk trade which includes small and medium capacity raw milk haulers

involved in wholesale or retail trading. The formal and organized sector which collects, processes and markets the rest of the produce (5 to 10%), and is largely conglomeration of corporate and multinationals, has established cold chain milk collection and transport system which is generally reported successfully operational (Fakhar and Walker, 2006). As the two are in competition it is generally blamed by the corporate sector that policy and practice of raw (unprocessed) loose milk retailing is both cause and effect of milk adulteration. Moreover, this bulk of milk is neither chilled nor standardized or tested at any stage of supply chain with the result that milk is suffering from all sorts of quality deterioration and adulteration; the purpose of latter is to conceal quality defects or to extend shelf-life. And it may be merely an undue profiteering by addition of extraneous water. Chemicals, used in milk and milk products as adulterants, are so numerous that it is almost impossible to enumerate or detect all of them simultaneously. Many workers have reviewed this problem with pros and cons on their detection and health implications of adulteration (Nayak and Bector, 1998; Kolhe et al., 2003; Unnikrishnan et al., 2005; Khan, 2006; Adam, 2008). These adulterants/contaminants directly affect the quality of milk and milk products and their nutrients (Afzal et al., 2011). Functional properties of important constituents such as protein are also changed resulting in deterioration in texture and appearance of the finished products. This practice may also prove highly deleterious in the long run for the consumers because long term exposure to even low concentration of apparently harmless chemicals may pose serious health hazards. Toxic effects of urea, at levels detected in adulterated milk, were investigated for in vivo chromosomal aberration test using the mouse bone marrow. It was concluded that the consumption of urea adulterated milk for a short duration (of even 7 days) can produce hepatotoxicity and nephrotoxicity; however, genotoxicity was not observed even after 28 days of the experiment (Kommadath et al., 2001).

It has been found that water is the most frequently and widely used adulterant (Rao et al., 2002; Abou-Dawoodet al., 2005; Aziz, 2006; Shaikh et al., 2013; 12). Unhygienic water employed for such purpose and poor milk handling practices, coupled with tropical climate, further increase bacterial load of milk (reducing nutrient content), including transportation and processing cost. The entire burden is shifted to the consumers who are ultimately deprived of due nutrients for which they are charged substantially and duly. Ice prepared from questionable bacterial quality water, is directly added for bringing milk temperature down to reduce bacterial multiplication in summer month. Indeed, it is just as like addition of chemical adulterants and warrants legal action. Concern regarding quality of loose as well as packed milk is sometimes projected in the section of print media but problem largely remains unaddressed because of unwatchful urban management (Shah, 1994; Lodhi, 1998).

Keeping in view gravity of the problem and widespread practice of adulteration a survey study was planned with the objective to ascertain the type of adulteration prevalence in the Pakistan capital (Islamabad) retail milk market and the seasonal pattern of incidence.

2. Materials and Methods

2.1. Sample Collection

Over a period of one year (2011), irrespective of type and source of milk, 280 raw milk samples were collected from the various markets of Pakistan capital, Islamabad, which were selected on random basis. A minimum of half a liter of sample (5 to 6 samples per week) was collected in virgin polyethylene pouches.

2.2. Analysis of Samples

All the samples were heated up to about 40°C (unless otherwise not required) and mixed thoroughly and latter cooled to the required/ ambient temperature before proceeding for

different types of test and evaluation. The entire range of tests, particularly quantitative ones, was carried out in duplicate according to standard methods described in and drawn from various sources [Ling, 1945; Judkins and Keener, 960; Atherton and Newlander, 1977; David, 1977, Watts et al., 1984; AOAC, 1990; FAO, 1991; APHA, 1992). Hydrogen peroxide was measured/detected by use of commercial strips, *Quantofix* manufactured by Macherey-Nagel GmbH & Co., Germany. The data was analyzed statistically using Minitab Computer Package.

Table 1. Percent composition of market milk in Islamabad milk market (2011)

Months	N	Fat	SNF	TS	Water
Jan	21	3.00±1.00	6.50±1.22	9.50±2.40	90.50±2.60
Feb	21	2.55±0.96	6.40±1.42	8.95±1.89	91.05±0.20
Mar	20	2.00±1.12	6.50±1.58	8.50±2.04	91.50±1.50
Apr	22	3.20±1.10	5.45±1.44	8.65±1.95	91.35±1.20
May	24	2.50±1.00	5.70±1.74	8.20±1.58	91.80±0.90
June	23	3.20±0.58	6.30±1.95	9.50±1.96	90.50±0.20
July	31	2.40±0.70	6.80±1.09	9.20±1.20	90.80±1.40
Aug	34	2.45±0.72	6.05±1.22	8.50±0.92	91.50±2.00
Sep	21	2.40±0.94	6.55±1.64	8.95±0.79	91.05±2.20
Oct	20	3.20±1.09	6.65±1.50	9.85±1.42	90.15±0.96
Nov	21	2.20±0.65	6.75±1.42	8.95±1.50	91.05±1.20
Dec	22	2.50±0.68	7.05±0.90	9.55±1.42	90.45±1.00
Overall	280	2.63±0.41	6.39±0.46	9.03±0.51	90.98±0.51
CV	--	38.21	24.59	21.46	2.22
Legal Standard	Buffalo	5.00	9.00	14.00	86
	Cow	3.50	8.50	12.00	88

Table 2. Month-wise frequency distribution of chemical adulteration of milk samples during 2011 in Islamabad milk market

Months	N	Number of samples adulterated with			Adulterated (%)
		Carbonate	Hydrogen peroxide	Starch	
Jan	21	Nil	Nil	Nil	Nil
Feb	21	1	Nil	Nil	4.76
Mar	20	1	2	Nil	15
Apr	22	5	2	Nil	31.82
May	24	11	2	2	62.5
June	23	11	4	2	73.91
July	31	15	3	3	67.74
Aug	34	21	3	3	79.41
Sep	21	5	3	2	47.62
Oct	20	2	1	1	20.00
Nov	21	2	Nil	1	14.29
Dec	22	1	Nil	Nil	4.55
Total (N)	280	75	20	14	
Positive (%)		27.00	7.00	5.00	

Table 3. Month-wise distribution of milk samples adulterated with water in 2011 in Islamabad milk market

Months	Added water according to	
	Buffalo milk legal standard (TS 14%)	Cow milk legal standard (TS 12%)
Jan	32±14	21±3
Feb	36±2	25±1
Mar	39±11	29±12
Apr	38±9	28±10
May	41±7	32±8
June	32±2	21±2
July	34±15	23±12
Aug	39±16	29±16
Sep	36±12	25±14
Oct	30±13	18±10
Nov	36±10	25±14
Dec	32±8	20±13
Overall	35.54±3.62	24.79±4.22
CV	41.15	53.45

Table 4. Frequency distribution of *added water* market samples (rounded to whole figures)

Part A (Buffalo milk legal standard, TS 14% (SNF 9 & Fat 5)

Water (%)	Legal Limit*	Up to 10	11 – 20	21 - 30	31- 40	41 - 50	> 51
Sample (N)	3	11	54	46	44	100	22
Sample (%)	1	4	19	17	16	36	8
Cum.%	-	4	23	40	56	92	100

*Water content not more than 86%

Part B (Cow milk legal standard, TS 12%, (SNF 8.5 & Fat 3.5)

Water (%)	Legal Limit*	Up to 10	11 – 20	21 - 30	31- 40	41 - 50	> 51
Sample (N)	34	47	39	38	91	26	5
Sample (%)	12	19	16	15	37	11	2
Cum.%	-	19	35	50	87	98	100

*Water content not more than 88%

3. Results and Discussions

The results are presented by grouping various observations under two main headings with relevant subheading or / and according to their relevance and relationship.

3.1. Milk composition

3.1.1. Total solids and fat

The total solids (TS-Table 1) or dry matter of milk and milk products are important because on one hand it is reflection of total nutrients and product quality and on the other hand yield of dairy products depends upon it. For certain products such as *Khoa* all the three components have equal importance.

The average of total solids was 9.03% with SD of 0.51 and overall CV 21.46. The average total solid were at least 35% less than minimum legal standard specific for buffalo milk according to "Pakistan Pure Food Laws" (Kazmi, 1983). But if this TS value is compared with real field (Athar and Ali, 1986) or farm values or authentic milk source values for buffalo milk (Rao, 2002) it would be almost half of such reference standards. This overall reduction in total solid is also verified from the calculation of water content. It can be concluded that the market retailer are habitual/ consistent in practice of adulteration with water. Since it appeared that high water content was result of willful act hence it has been dealt in detail below.

3.1.2. Added water

The amount of added water (Table 1, 3 and 4) has been calculated by the following formula as given by APHA, 1992 and modified by Khattak (1999):

$$\text{Added Water (\%)} = [\{\text{TS (legal standard)} - \text{TS (sample)}\}\ 100] / \text{TS (legal standard)} \quad (1)$$

The results are shown with a frequency distribution in the Tables 3 and 4. With respect to buffalo milk (containing 14 % as minimum legal TS) it was found that hardly three samples conform to legal minimum TS otherwise all the samples were found watered, from 10 to 50 percent. Majority of the samples (36%) contained added water between 41% and 50%. Even 8% samples contained more than 50% of added water which mean reducing the total solid less than half of what is normally supposed to be present. The average added water contents with reference to cow milk standard were 24.79% with standard deviation of 4.22 (Table 3). The CV % was about 53.45. This reflects huge variation of added water in the samples without significant difference between the months.

With respect to cow milk (minimum legal standard being 12%) the situation is better because of lower legal limits. In this case, 12% of the samples conformed to minimum criterion of cow milk for TS content. The rest of the samples (88%) contained added water ranging from 10 to 50% and about half of them (46%) contained added water ranging from 21% to 40%.

When total solids were compared with the legal standard according to "Pakistan Pure Food Laws" it was revealed that 99% of the samples failed to confirm buffalo milk criteria and 88% failed to conform cow milk criteria, and majority of samples contain extraneous water between 40 and 50%. Sale of such poor quality milk openly is clear reflection of noncompliance of legal standard by retailers. Persistency and magnitude of added water in the samples speaks well about the negligence of local legislature and administration. It appears that there is no regular quality monitoring activity of dairy products in practice; this view is supported further by the number of market milk samples, on average almost 50 each month, being analyzed by the public analyst (Amjad, 2007).

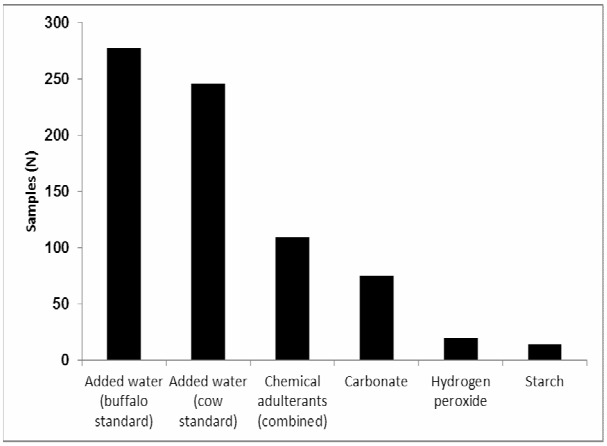

Figure 1. Frequency distribution of some adulterants detected in milk samples of Islamabad market during 2011.

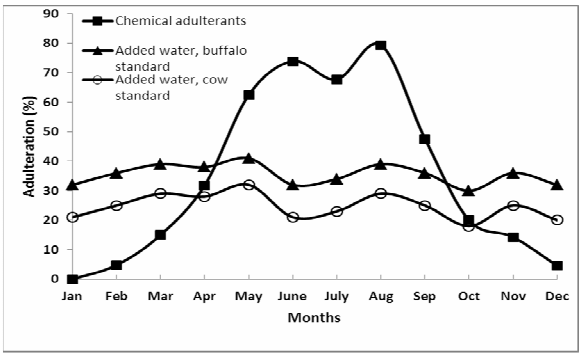

Figure 2. Monthly trend of milk adulteration in Islamabad milk market (2011)

3.2. Adulteration

3.2.1. Added/Extraneous Water

In the study under report the added water was found as the most frequent adulterant both in level of practice and incidence. It seems that this is the single most widely used adulterant in the loose milk retailing. It has been reported by number of workers that as the supply chain moves towards consumers, the proportion of extraneous water increases steadily by a factor of 10 to 50 percent (Aziz, 2006; Khattak, 1999; Faraz et al., 2013).

The water content of normal milk varies from 82% to 87%. Any variation in other constituents is also reflected upon the water content. In this study the average water contents were 90.98% with standard deviation of 0.51 and CV only 2.22%. There was no significant difference between the months. From this small CV, a generalization can be made that water content of market milk would fall above 90% for almost all the available /sold milk.

3.2.2. Chemical adulterants

The chemicals commonly reported as adulterants in milk are far numerous to mention (Kolhe et al., 2003; Khan, 2006; Azis, 2006; Barham et al., 2014). However, the most common in practice are carbonate / bicarbonate, hydrogen peroxide, urea, starch etc. Sometime, even formalin has been reported from dairy products, but only very occasionally (Abou-Dawood et al., 2005; Barham et al., 2014). Adulterants and chemicals are usually used to conceal defects or to improve upon deteriorated milk characteristics or tone up certain milk quality indices.

a. Hydrogen per oxide (Table 2, Figure 1)

This is also an important and popular milk adulterant due to its antibacterial activities. It is usually used in harsh summer season to extend the shelf-life of microbiologically pure quality milk through out the entire developing countries. Twenty samples (7%) were found adulterated with this adulterant; the incidence was particularly high in summer months of June, July and August. In a recent study in Egypt 3.3% cow's milk samples were found to contain hydrogen peroxide (Mansour et al., 2012). In Pakistan 13% of the samples collected from Mirpurkhas district were found adulterated with hydrogen peroxide (Barham et al., 2014), whereas in Faisalabad district 3% of the samples were found to contain hydrogen peroxide (Faraz et al., 2013).

b. Carbonate (Table 2, Figure 1)

Carbonate is the most typical popular chemical which fulfills all the requirements of a

"preservative" and at the least possible cost. Not only this neutralizes the developed acidity (lactic acids) which is result of rapid bacterial multiplication but also extends the shelf life of milk and can fool otherwise a reliable criterion such as COB test. Out of 280 samples analyzed 75 samples were found adulterated with carbonate. This is the only chemical adulterant, apart from added water, which was found most frequently in the market samples. The samples were abnormal in taste that is salty. Carbonate is the frequently employed adulterant to neutralize any developed acidity in hot and humid areas where cold chain facility is not available due to any reason. In a similar study 48% of the market milk samples collected from Hisar, India were found adulterated with carbonate (Garg and Mandokhot, 1997). In Egypt 3 out of 60 (5%) examined milk samples were positive for carbonate/bi-carbonate whereas 4 out of 60 collected from vendors were positive for carbonate and bi-carbonate (Mansour et al., 2012).

c. Starch (Table 2, Figure 1)

Starch is reported to be used as an adulterant for improving the viscosity of the milk thereby enhancing the consumer acceptance as regards visual and gustatory impressions. Among the samples tested 14 samples (5%) were found adulterated with starch. In a similar study conducted in Mirpurkhas district, Pakistan 12% of the milk samples were found to be adulterated with starch (Barham et al., 2014). In Khartoum State in Sudan none of the samples analyzed were found to contain starch (Adam, 2009). The reason of the low incident is that when diluted milk is equally well saleable why worry to improve upon apparent defects.

4. Conclusions

The next conclusions can be drawn:

(i) Although database is small yet it is evident that high temperature season, i.e. summer is the period of maximum adulteration observed from May, when adulteration suddenly shot and remained more or less consistently high in June to August and then started declining till it touched rock bottom in winter months.

(ii) Added water is the most frequently founded adulterant in the market milk.

(iii) Carbonate, followed by hydrogen peroxide and starch are major chemical adulterants found in the market milk.

(iv) The compositional quality of milk was significantly poor as compared with the acceptable limits of even cow milk.

(v) Incidence of chemical adulteration is almost negligible in winter season. This indicates that *lack of chilling* facilities and *high milk demands* in summer season are two principal factors to resort for adulteration.

(vi) The overall incidence and severity of problem is much less as compared with many Indian Milk Markets.

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CAROTENOID ANALYSIS IN FOOD

Luiza-Mirabela Ciuca[1*], Cristina Mihali[1]

*[1]Department of Science, Technical University of Cluj Napoca, North University Center of Baia Mare, Victoriei 76, 430122, Baia Mare, Romania; *luiza_ciuca@yahoo.com*

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ABSTRACT
The aim of this study was to analyze the main types of carotenoides in carrots, spinach, tomatoes, tomato products and in margarine. The carotenoids composition of foods vary from the qualitatively and quantitatively point of view. We have qualitatively determined the carotenoids in the above mentioned food types by open column chromatography. The results showed the presence of lutein in spinach, of lycopene in tomatoes and in tomato products and of β-carotene in carrots, spinach and in margarine.

1. Introduction

Carotenoids are isoprenoid molecules that are widespread in nature and are typically seen as pigments in many flowers, fruits and vegetables (Rodriguez-Amaya and Kimura, 2004). They contribute to the red, orange and yellow colors found. Are indispensable to plants and play a critical role in human nutrition and health. Because plants are able to synthesize carotenoids *de novo*, the carotenoid composition of plant-derived foods is enriched by low levels of biosynthetic precursors and derivatives of the main components (Leonte, 1998). Most fruits and fruit vegetables have higher carotenoid levels in the peel than in the pulp. For all the processing methods, the degradation of carotenoids increases with prolongation of processing time, higher processing temperature, and cutting of the food (Rodriguez-Amaya and Kimura, 2004).

Food samples typically contain both the non-polar carotenes and the more polar xanthophylls. Whatever the method used, the chromatographic process should be able to cope with this polarity range. Thin-layer chromatography (TLC), although efficient in monitoring the progress of chemical tests for identification purposes, is not adequate for quantitative analysis because of the danger of degradation and isomerization on a highly exposed plate (Lu and Li, 2008).

Most of the qualitative and quantitative data, generated in our laboratory, suggest that different classes of carotenoids differ in their stability towards heat treatment. For example, on the effects of cooking and processing on a number of yellow/orange vegetables has revealed that the destruction of the hydrocarbon carotenoids such as α- and β-carotene as a result of heat treatment is about 8- 10%. Carrots do not contain esterified carotenoids and have low lipid content; hence saponification is unnecessary. (i) Leafy vegetables produced in greenhouses or in plots

covered with plastic wrap present higher concentrations of carotenoids in the summer. However, carotenoid levels of the leafy vegetables grown in the open are significantly higher in winter than in summer, suggesting that photodegradation is more intense than carotenogenesis. Carotenoid biosynthesis can occur in fruits, vegetables, and root crops, even after harvest, provided that the plants are kept intact, preserving the enzymes responsible for carotenogenesis. (ii) The leaves and other vegetables, post harvest carotenoid degradation may predominate, especially at high temperatures and in the storage conditions favoring brown rot. (iii) Carotenoids are naturally protected plant tissues but processes as cutting, breaking, kicking and peeling fruits and vegetables increase the exposure to the action of oxygen and favor the oxidation reaction between carotenoids and enzymes (Lu and Li, 2008; Rodriguez-Amaya, 2001). (iv). Stability of carotenoids differs in different foods, even when using the same processing and storage conditions. The optimal conditions for retention carotenoids during the processing are different from one food to another. Different carotenoids have different susceptibilities to degradation. (v) The main cause of the destruction of carotenoids during processing and storage is enzymatic and non-enzymatic oxidation. Isomerization of trans-carotenoids in cis-carotenoids, in particular during the heat treatment alters the activity and modifies food color, but not to the same extent as oxidation. In many foods, the degradation enzymatic carotenoid may be higher than thermal decomposition. (vi) Deep pan, cooking prolonged combination preparations and different methods of cooking, baking and pickling, all resulting substantial loss of carotenoids. (vii) For these curves, the purity of the standards was 98% for lutein, 97% for zeaxanthin, 96% for β-cryptoxanthin, and 98% for β-carotene.

The aim of this study is to identify the concentration on carotenoids in carrots, spinach, tomatoes and in margarine. Also, we observed the impact of heat treatment of processing on the caroteinoid composition.

Lycopene is present in high concentration in mature tomatoes and in tomato paste is lost gradually by heat treatment. Margarine had to undergo saponification process to remove the fatty acids present and to extract carotene (E160a). In margarine, the carotene content is little being just food coloring.

Cooling (in particular, the rapid cooling) and freezing processes are generally conserved carotenoids. Instead slow thawing can reduce the content of carotenoids.

2.Methods

2.1 Sampling

To obtain meaningful and reliable analytical data, the sample must be representative of the entire lot under investigation and adequately prepared for analysis. The more heterogeneous the material, the greater the difficulties and effort required to obtain a representative sample. Because food samples are typically heterogeneous, a large number of samples should ideally be analyzed. In practice, however, the sampling procedure adopted is usually a compromise between heterogeneity considerations and the cost of the operation (Rodriguez-Amaya and Kimura, 2004; Rodriguez-Amaya, 2001).

Carrots, spinach leaves, tomatoes were taken from the food market. Frozen spinach, tomato paste and margarine were purchased from the hypermarket.

2.2 Sample preparation

Two pieces of carrot roots (*Daucus carota)* were washed and dried with an absorbent paper and then were cut into small pieces. This operation was realized rapidly to prevent enzymatic degradation of the carotenoids. The sample was homogenized. The homogenization and sub-sampling was realized simultaneously or consecutively in either order.

Spinach leaves were washed and left to dry. They were then broken by hand into small pieces. Tomatoes were also washed and dried. Tomato sample included both core and skin.

Tomato paste was taken directly from the preserve. Margarine was bought from the supermarket. Further we used open column chromatography (OCC) with silica gel as stationary phase.

2.3. Extraction

A good extraction procedure should release all the carotenoids from the food matrix and bring them into solution, without altering them. Because they are found in a variety of foods, the extraction procedure should be adapted to suit the food being analyzed.

An amount of 5 g small pieces of carrots were mixed with 10 g sand and 10 g anhydrous Na_2SO_4. The extraction was realized in a mortar in the presence of a mixture of acetone and petroleum ether (1:1, v:v). The extraction liquid was filtered and was diluted with distillate water. Thus two layers were obtained as follows: (i) superior-ether layer which contains the carotenoid pigments; (ii) inferior-layer formed by water and acetone. Extraction and filtration are repeated until the residue is colorless (three extractions are usually enough).

A number a 10 fresh spinach leaves were used later to weigh 5 grams of crushed sample. Tomatoes (5 g) were chopped and mixed with 10 grams of sand for a better extraction. An amount of 10 g tomato paste was also used. The three samples were processed similar with the carrots using petroleum ether and acetone as the extraction reagents. For tomato paste, frozen spinach and margarine were used different processes. The distribution of the carotenoids is presented in Figure 1.

The margarine sample was first subjected to the saponification reaction to extract the carotenoids (Figure 2). Saponification is an effective means of removing chlorophylls and unwanted lipids, which may interfere with the chromatographic separation and shorten the column's life. Into a flask of 300 ml, an amount of 5 g margarine was introduced. Then, a volume of 100 mL ethanol and 30-40 mL solution KOH 15 % were added and the mixture was refluxed for 20 min. The saponification can be performed in cold conditions and the mixture was let over night.

After the finish of the saponification process (and cooling), the mixture was transferred in a separator funnel of 500 mL and 75 mL petroleum ether was added. The mixture was stirred for 10-15 minutes and slowly was added 20-30 mL water for the separation of the phases. The etheric phase was washed with portions of 40-50 mL distilled water until the neutralization of aqueous phase.

After complete removal of the last wash water transfer etheric solution in a balloon and evaporate at dry 50-60 °C. Over the residue obtained, a volume of 20 to 30 mL hexane was added, measuring the exact volume in a flask.

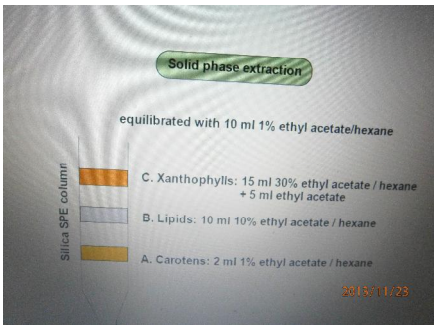

Figure 1. The distribution of carotenoids

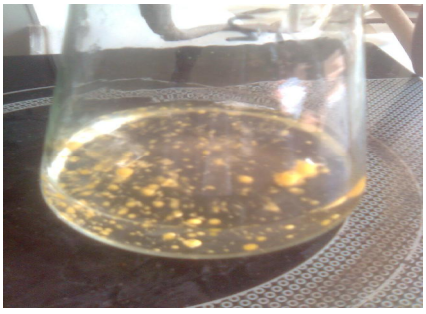

Figure 2. Macro-view of the mixture during the margarine saponification

A better extraction of beta carotene when using a higher temperature has been observed. Xanthophylls degrade very quickly, so I took care to solvents. A system to reflux was used and gradually formation of fat cells of mixture with beta-carotene was noticed. They were collected in a separating funnel with fine pores.

2.4. Partition

A volume of 1 mL sample extract together with 20 mL petroleum ether (PE) was introduced in a separator funnel. A volume of extract was added. After each addition, a volume of 300 mL distilled water was added slowly, letting it flow along the wall of the funnel.

To avoid formation of an emulsion, do not shake. Let the two phases separate and discard the lower, aqueous acetone phase. Add the second portion and repeat the operation. After the third portion has been transferred to PE, washed 3 times with water (about 200 mL distilled water, let the phases separate, and discard the lower phase) to remove residual acetone. In the last washing, be sure to discard the lower phase as completely as possible, without discarding any of the upper phase. It (Figure 3) was collected in a 25 mL volumetric flask and was passed through a funnel with anhydrous sodium sulfate to remove residual water. The funnel was washed with a small amount of PE, collecting the washings into the volumetric flask. The extract usually contains a substantial amount of water, which can be removed by partitioning to hexane, petroleum ether, diethyl ether, or dichloromethane, or mixtures of these solvents. Diethyl ether or a mixture of this solvent with hexane or petroleum ether is preferred for extracts with large amounts of xanthophylls, part of which is lost during partitioning with pure hexane or petroleum ether.

Figure 3. The partition of carotenoids in carrot sample

2.5. Chromatographic separation

With a pipette, add the carotenoid PE solution into the column and let the sample layer go down almost to the surface of the sodium sulfate layer before adding the rings (PE) from the round bottom flask, the objective being to keep the carotenoids in as small a volume as possible to diminish band broadening and to prevent the separation from initiating before the entire carotenoid sample has reached the top of the adsorbent. Develop the column, adjusting the mobile phase so as to isolate the desired carotenoids as quickly and efficiently as possible. Elute α-carotene with PE, β-carotene with 2% acetone, β-cryptoxanthin with 15–20% acetone, lutein with 25–30% acetone, and zeaxanthin with 40–45% acetone in PE. For α-carotene and β-carotene, leave the other carotenoids in the column after elution of these carotenoids (Figure 4). For β-cryptoxanthin, lutein, and zeaxanthin, discard the carotenoids that elute from the column before these xanthophylls.

As acetone affects the absorption of carotenoids in PE, remove the acetone from the β−carotene, β−cryptoxanthin, lutein, and zeaxanthin by washing with water in a separatory funnel. Dry the PE solution of the carotenoid with anhydrous sodium sulfate.

Figure 4. Chromatographic separation in open column

2.6. Construction of Standard Curves

The calibration curve was obtained from β-carotene pills. Due to similar absorbtion proprieties, as standard solution could be used a

potassium dichromate solution. In this purpose, 300 mg of potassium dichromate dried and recrystallized (3 times) was dissolved in distilled water in the flask of 1 dm^3. A volume of 1 cm^3 of solution corresponds to 0.00208 mg (2.08 µg/cm^3) of carotene.

Based on the results obtained from the calibration graph we calculated the amount of carotene mg in 100 g of sample. Dilutions were made and the extinction at 448 nm was measured using the PG Instruments spectrophotometer. The curve should pass through or very near the origin, be linear with a correlation coefficient ≥ 0.95, and should bracket the concentrations expected in the samples. The calibration curve is presented in Figure 5.

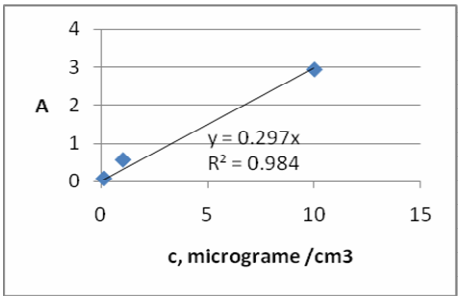

Figure 5. The calibration curve of carotene

3. Results and discussions

The content of lycopene and beta-carotene in tomatoes was 10 µg/g and 7 µg/g, respectively. In the tomato paste, the content of lycopene was 8.8 µg/g and that of beta-carotene was 6.5 µg/g.

The content of beta-carotene in the analyzed margarine was 610 µg/100 g. Carrots contain 8,828 mg/100g β-carotene, 3,477 mg/100g α-caroten, 4 µg/100g lycopene and 350 µg/100g. β-cryptoxanthin was not found. The spinach contains 5.625 mg/100 g β -carotene and the frozen sample contains 3.4 µg/100g β -carotene.

4. Conclusions

The next conclusions can be drawn:

(i) The degree of crushing greatly influences carotenoid extraction, and solvent used.

(ii) In the preparation of food at home, the loss of carotenoids takes place in the following way: microwave <steam <boil <roasting.

(iii) Leaves have a strikingly constant carotenoid pattern, often referred to as the chloroplast carotenoid pattern, the main carotenoids being lutein (about 45%), β-carotene (usually 25–30%), violaxanthin (15%), and neoxanthin (15%).

(iv) α-carotene and β-carotene are predominant in carrots.

(v) The color of fruits and vegetables is given as we have seen by the presence of carotene: yellow-orange in carrots due to the presence of α and β-carotene, lycopene in tomatoes, spinach green because chlorophyll and beta carotene.

(vi) Temperature and harvest time significantly influence the carotenoids concentration of tomatoes paste and spinach frozen.

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EFFECT OF PACKAGING ON QUALITY OF MINIMALLY PROCESSED FENNEL

Miceli Alessandro[1]*, Moncada Alessandra[1], Vetrano Filippo[1], D'Anna Fabio[1]

[1]Dipartimento di Scienze Agrarie e Forestali, Università di Palermo, Viale delle Scienze, Palermo, Italy
** alessandro.miceli@unipa.it; Tel +3909123862219; Fax +3909123862240Viale delle Scienze 90128 Palermo, Italy*

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ABSTRACT
Fennel is not a widespread product among minimally processed vegetables, due to the fast deterioration mainly caused by browning of the cut surfaces, even during cold storage. In order to extend the shelf-life of fresh-cut fennel, the influence of different packaging techniques was studied. Sliced fennel, dipped in citric acid solution (0.5 %), was placed in polystyrene trays, sealed with PE film or vacuum packaged in PE bags and stored up to 14 days at 4°C. During this period weight loss, soluble solid content, pH, colour and firmness were evaluated. Minimally processed fennel packed in sealed trays and cold-stored at 4°C, extended its shelf life to 14 days maintaining acceptable quality. Fennel stored in vacuum packaging showed a lower weight loss but had severe alterations of flavour that led to loss of marketability after only 7 days.

1. Introduction

The demand for fennel (*Foeniculum vulgare* Mill.) has increased in many European countries such as Germany, Italy, The Netherlands and United Kingdom. It can be eaten cooked or as a fresh vegetable. Fennel production in Italy is quite large, especially in Central and South Italy, where the harvest season extends mainly from November to May, with a yield of about 40-45 t ha^{-1}. Fennel bulbs are harvested when they have an equatorial diameter greater than 6 cm, ensuring that the bulbs are firm, white, sweet, and without damage. Usually fennel bulbs are not cold-stored for a long time, being harvested according to market demand. The major post-harvest losses of fennel under high humidity and low temperature conditions are due to weight loss and decay associated with bacteria and moulds (Escalona et al., 2006).

Fennel is not a widespread product among minimally processed vegetables, due to the fast deterioration even during cold storage. Browning on the butt-end cut zone of the bulb or in the cut surfaces of minimally processed fennel is the most important cause of quality loss during storage and distribution (Albenzio et al., 1998; Artès et al., 2002a). Quality and shelf-life of minimally processed vegetables may be affected by pre-harvest (Miceli and Miceli, 2014; Settanni et al, 2012; Settanni et al, 2013) or post-harvest (Miceli et al., 2013) factors, thus raw material quality, post-harvest treatments and packaging can greatly influence consumer acceptance and produce marketability.

In order to extend the shelf-life of minimally processed fennel, the influence of different packaging techniques was studied.

2. Materials and methods

'Pontino' fennel was field-grown in Castelvetrano (Trapani, Italy) in the south-west coastal area of Sicily. Bulbs were hand-harvested in the second half of December, selected in field, eliminating the soiled and decayed external leaves, and directly transported to the laboratory and stored at 4°C.

After the removal of the inedible parts, fennel hearts were washed with chlorinated water (100 ppm) for 10 min. Each bulb was then cut in 8 slices of 30-40 g. Immediately after cutting, the slices were immersed in chlorinated water (100 ppm) for 5 min, rinsed to lower the free chlorine, dipped in a 0.5% citric acid solution for 5 min and then centrifuged in a manual salad spinner to remove excess water. Samples of 100 g were immediately placed in polystyrene trays sealed with PE film or vacuum packaged in PE bags and stored up to 14 days at 4°C. During this period samples of each packaging were taken (on day 0, 7 and 14) and weight loss, colour, firmness, soluble solids, pH and overall quality were evaluated.

Weight loss was evaluated by weighing samples after processing and during storage. The results were expressed as grams per 100 g of initial fresh weight.

Colour changes were measured with a colorimeter (Minolta chroma-meter CR-400) by measuring parameters L*, a* and b* at 2 points on the external leaf of three slices and at 2 points of the cut zone of three slices on day 0, 7 and 14 of storage at 4°C. Hue angle (h°) and Chroma (C*) was calculated as $h° = \arctan(b^*/a^*)$ when $a^* > 0$ and $b^* > 0$, or as $h° = 180° + \arctan(b^*/a^*)$ when $a^* < 0$ and $b > 0$ (McLellan et al., 1995) and $C^* = (a^{*2} + b^{*2})^{1/2}$.

Firmness was determined by taking an average of 4 readings from the external leaf of the slices for each sample using a digital penetrometer with an 8 mm diameter stainless steel cylinder probe.

An informal panel made of seven people (4 men and 3 women, aged 25-45), familiar with the sensory qualities of fennel, evaluated appearance, aroma and texture. From these properties, overall quality (OQ) was measured using a 1 to 5 scale, with 5 = excellent or having a fresh appearance and typical taste and firmness, 3 = fair/limit of marketability, and 1 = poor /unmarketable with great alteration in texture or in taste.

Samples were then juiced with a commercial home juicer and total soluble solids (°Brix) and pH were determined using respectively a digital refractometer and a digital pH-meter.

A completely randomized design with three replications per treatment was performed. To determine the effect of storage time and packaging a two-way ANOVA was carried out. Mean values were compared by the LSD multiple range test to identify significant differences among treatments and significant interactions between factors.

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3. Results and discussions

Minimally processed fennel was stored for 2 weeks at 4°C. During this period weight loss was influenced both by time of storage and by packaging (Table 1). Fennel stored in vacuum packaging showed a very low weight loss (< 1%) after 14 days of storage, while fennels in trays reached 1,66% after only 7 days and 1,82% after 14 days. Nevertheless, the weight loss did not cause shrinkage and drying of sliced fennels packed in trays.

Packaging had no influence on firmness (Table 1). Storage time was the main factor affecting the softening of the external leaves. After processing, firmness was 60.81 N; similar values have been reported, for whole fennel at harvest, by Artés et al. (2002b). During storage at 4°C firmness decreased on average to about 4 N after 7 days and to 11.46 N after 14 days. No significant interaction between time of storage and packaging was found.

Soluble solids content decreased during storage period with no differences between tested packaging (Table 1). The reduction of SSC was, on average, of 0.89 °Brix after 14 days of storage. Many authors have reported reduction of sugar content during cold storage of fresh cut vegetables. According to them, sugars loss could be mainly due to hydrolysis of sucrose toward glucose and fructose (the predominant sugars in fennel) and their consumption for maintaining the energy requirements of the cells (Escalona et al., 2005, 2006; Blanchard et al., 1996; Heimdal et al., 1995).

Table 1. Changes in weight loss, firmness, soluble solids content (SSC), pH and overall quality (OQ) of minimally processed fennel during storage at 4°C in different packaging.

Time (day)	Packaging	Weight loss (%)	Firmness (N)	SSC (°Brix)	pH	OQ
0			60.81 a	5.70 a	6.23 d	5.00 a
7	Tray	1.66 B	57.41 ab	5.20 b	6.31 c	3.83 b
	Vacuum storage	0.30 D	56.41 b	4.97 b	6.80 b	2.17 d
14	Tray	1.82 A	48.53 c	4.83 c	6.29 c	3.00 c
	Vacuum storage	0.70 C	50.16 c	4.80 c	6.87 a	1.17 e
Time		***	***	***	***	***
Packaging		***	ns	ns	***	***
Time x Packaging		***	ns	ns	***	***

Data within a column followed by the same letter are not significantly different according to LSD multiple range test. Probability: ns not significant; * significant at P<0.05; ** significant at P<0.01; *** significant at P<0.001

Table 2. Colour changes of external leaves during storage at 4°C in different packaging.

Time (day)	Packaging	L*	a*	b*	Chroma	Hue angle
0		86.44 a	-2.86	12.27	12.60	166.87 b
7	Tray	83.96 b	-2.69	10.61	10.95	165.79 b
	Vacuum storage	83.05 b	-2.88	11.69	12.05	165.96 b
14	Tray	83.26 b	-2.78	10.46	10.82	165.10 b
	Vacuum storage	79.16 c	-2.37	12.78	13.01	169.37 a
Time		***	ns	ns	ns	ns
Packaging		***	ns	*	*	*
Time x Packaging		**	ns	ns	ns	*

Data within a column followed by the same letter are not significantly different according to LSD multiple range test. Probability: ns not significant; * significant at P<0.05; ** significant at P<0.01; *** significant at P<0.001

Table 3. Colour changes of cut zones during storage at 4°C in different packaging.

Time (day)	Packaging	L*	a*	b*	Chroma	Hue angle
0		80.76 a	-2.59	14.84	15.07	170.14
7	Tray	79.28 b	-2.38	10.53	10.79	167.27
	Vacuum storage	77.02 c	-2.84	11.97	12.30	166.70
14	Tray	78.42 b	-2.56	11.75	12.02	167.68
	Vacuum storage	75.94 c	-2.51	11.10	11.38	167.28
Time		***	ns	***	***	***
Packaging		**	ns	ns	ns	ns
Time x Packaging		*	ns	ns	ns	ns

Data within a column followed by the same letter are not significantly different according to LSD multiple range test. Probability: ns not significant; * significant at P<0.05; ** significant at P<0.01; *** significant at P<0.001

In both packaging, pH increased during storage (Table 1). The pH of fennel packed in trays increased only during the first week, while vacuum storage determined a higher value after 7 days and continued increasing until the end of storage with a significant interaction between treatments (Table 1).

Colour changes of external leaves are reported in table 2. Parameter L* showed a decrease during storage. The lightness (L*) of the external leaves of fennel stored under vacuum, progressively decreased during 14 days of storage (from 86.4 at the beginning of storage to 79.16) while fennel packed in trays had no further decrease after 7 days of storage. The other parameters of colour were not affected by the duration of storage. Vacuum storage did not show any significant influence on a*, b* and chroma, but determined an increase of hue angle after 14 days (169.37). On the contrary the fennel stored in polystyrene trays had lower values of b* and chroma, with no modification of hue angle.

Time of storage and packaging influenced the lightness of cut zone (Table 3). The L* value decreased during the first week of storage, after which no further changes occurred. The decrease was larger for vacuum packed fennel than for those packed in trays (respectively 75.94 and 78,42 after 14 days of storage). The main factor that affected b*, chroma, and hue angle values was the duration of storage. No changes were noted for a* values. The decrease of hue angle and the larger reduction of lightness of cut zone of vacuum packed sliced fennel corresponded to a more severe browning than tray packed fennel. Browning was not visible in vacuum packed fennel, but developed rapidly once the packages were opened.

Overall quality was evaluated considering both visual quality (appearance and browning) and sensory quality (aroma, taste, odour, texture). Storage period affected the loss of quality but the major effect was due to packaging (Table 1). General quality of minimally processed fennel stored in polystyrene trays was mainly reduced by changes in colour and weight loss. Nevertheless, after 14 days of storage in polystyrene trays, fennel slices were above the limit of marketability. Vacuum packaging determined a more severe reduction of overall quality. Fennel slices were below the threshold of acceptance only after 7 days of vacuum storage due to enzymatic browning that appeared once the package was opened and due to unfavourable odour and altered flavour. Probably the very low O_2 level and the high CO_2 level led to undesirable responses as the induction of fermentation, the development of disagreeable flavours, a reduction in aroma biosynthesis, the induction of tissue damage and an alteration in the makeup of microbial fauna (Beaudry, 1999). Albenzio et al. (1998) reported that vacuum packaging can promote anaerobic bacteria development like Vibrionaceae or Enterobacteriaceae. They can develop even during cold storage, fermenting glucose and producing substances that can alter flavour and odour.

4. Conclusions

Minimally processed fennel packed in sealed trays and cold stored at 4°C, can extend its shelf life to 14 days maintaining acceptable quality. Vacuum packaging and cold storage determined a lower weight loss and a better visual quality of packed fennel but the extreme conditions of atmosphere (very low O_2) caused severe alterations of flavour that led to loss of marketability after only 7 days. The role of microbiological contamination in altering fennel quality during vacuum storage needs further studies.

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MIXTURE OF SOLID WITH WATER SOLUBLE, OLIVE OIL MILL WASTE APLICATION, AS SOIL AMENDMENT IN GREENHOUSE CULTIVATION OF VEGETABLES (CASE STUDY)

Nikolaos Gougoulias[1*]; Ioannis Vagelas[1]; Alexandros Papachatzis[1]; Dimitrios Kalfountzos[1]; Liviu Giurgiulescu[2], Adamantia Chouliara[3]

*[1] Department of Agronomy Technology, Technological Educational Institution (TEI) of Thessaly, 41110 Larissa, Greece; *ngougoulias@teilar.gr*

[2] Department of Chemistry and Biology, Technical University of Cluj Napoca, North Universitary Center of Baia Mare, 430122 Baia-Mare, Romania

[3] Informatic's Scientist, data analysis

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ABSTRACT

In greenhouse cultivation with spinach and endive, the effectiveness of implementation of mixture of solid and water soluble olive oil mill waste, in replacement of mineral base dressing, was tested. The mixture of olive mill waste used, was constituted in the ratio of (1solid:3 water soluble); the used mixture correspond to 21 tons of water soluble waste and 7 tons of solid waste per hectare, or 17.34 kg of N, 22.63 kg of P_2O_5 and 98.7 kg of K_2O per hectare; though, the needs of the plants, were satisfied with complementary mineral addition as top dressing, via irrigation. The soil electrical conductivity during the cultivation was maintained at relatively low levels, with values ranging from 0.25 to 0.64 dSm^{-1} (water extract, 1 soil: $5H_2O$). The study showed that the incorporation of that mixture to soil, is an efficient amendment for greenhouse soil with vegetable crops; at the end of the growing period, soil salinity was maintained at low levels, no increase of heavy metals was observed, and the crop returned satisfactory yield. Furthermore, the recycling of that organic waste on crops, constitutes a very important solution for environment protection.

1. Introduction

Very important amounts of olive mill waste are produced in olive cultivation areas, ranging between $1.75x10^6$ and $2.25\ x10^6$ tons/year of water-waste for Greece, (Kyriazopoulos et. al., 2005), then use of these organic materials can be beneficial both to soil improvement and environmental protection. A satisfactory content in organic matter in the soil, dominates positively the soil fertility, (Chouliaras et al., 1998, Gougoulias et al., 2010), and the two-phase olive mill waste application to olive grove soil, increased organic carbon, total N, available P & K, and increased also olive production, (Lopez-Pineiro et al., 2008).

In a previous work, with incubation experiment, solid and water soluble olive oil mill waste, at different mixtures with the soil, showed that the organic content of water soluble olive oil mill waste, is subjected to high rate of biodegradation but solid waste, showed a strong resistance to biodegradation; nevertheless, a mixture of the two kinds of waste at a ratio of solid/liquid (1:3), added to soil, even the organic substance of solid phase, showed an increased biodegradation of about 40% (Gougoulias et al., 2013).

The purpose of this work, is the study of application of a mixture of solid and water soluble olive oil mill waste with ratio of (solid :

water soluble = 1:3) in a greenhouse with vegetable crop; the monitoring of the nutritional evolution of the crop, is by measuring the soil electrical conductivity (Gougoulias et al., 2012); in according to previous works, the increased electrical conductivity in soil extracts, is related with the increased concentration of soluble N and K; nevertheless, the concentration of phosphorus in soil, is not significantly affecting salinity (Chouliaras et al., 1991). Soil salinity assessment is based on measurement of soil electrical conductivity, reliable and easy method that could be used during cultivation period, for indication of soil fertility in a greenhouse crop (Rhoades et al., 1999).Then, soil electrical conductivity (EC) values, resulting from soil chemical composition, water irrigation quality used, and previous cultural techniques adopted, can be taken into account to plant fertilizer application; in according to that policy, as it concerns the studied greenhouse, EC value about 0.4 dSm^{-1}, (water extract of 1soil: 5 H_2O), is a criterion either to omit base dressing, or to stop surface fertilizers application as top dressing.

2. Materials and methods

In the greenhouse of the TEI of Thessalia, situated at Larissa (Greece), an area of 100 m^2, with Sandy Loam soil (table 1), slightly calcareous, with alkaline pH, low content in organic matter and high cation exchange capacity, was enriched before sowing, with 280 kg of a mixture constituted by 70 kg of solid, and 210 kg of water soluble waste, (Table 2); the waste mixture, was incorporated efficiently and seeding followed that application. The mixture with ratio (solid waste: liquid waste) was 1:3 namely, it corresponds to 7 tons of solid waste, and 21 tons of water soluble waste addition per hectare, altogether corresponding to 2.56 tons of organic matter addition to hectare.

Solid and aqueous olive waste is produced through three-phase decanter process from an olive mill located in Larissa. The solid wastes or olive husk is a mixture of olive pulp and olive kernel, while the aqueous olive waste (olive mill waste water), is the water added during preparation and oil extraction process (olive fruit washing, malaxation and centrifugation). One three –phase centrifuge type oil mill produce approximately 500 kg wet solid waste and 1200 kg water soluble olive mill waste, derived from 1000 kg processed olives.

Soil nutrients: Thereafter in an area of 100 m^2, sowed with spinach variety viroflay (50 m^2) and endive variety escarole (50 m^2) on 23-10-2013, the study lasted 93 days after seeding. By measurement of electrical conductivity (EC) of the soil extract (soil : water = 1:5) in samples from soil surface during crop growing, soil salinity was systematically controlled every week, and according to salinity level, the nutritional status of soil is satisfactory approached (Gougoulias et al., 2012). On the 9^{th} day after sowing, EC was found at the value of 0.25 dS m^{-1}, then nutrients added as top dressing, with (20-20-20) and (13-0-38) fertilizer types, via irrigation water, in amounts of 23, 10 and 48 Kg/ha respectively of N- P_2O_5 and K_2O.

Samples were analyzed using the following methods which are referred by (Page et al., 1982 and Hesse et al.,1972). Organic matter was analyzed by chemical oxidation with 1 $molL^{-1}$ $K_2Cr_2O_7$ and titration of the remaining reagent with 0.5 $molL^{-1}$ $FeSO_4$. Both ammonium and nitrate nitrogen were extracted with 0.5 mol L^{-1} $CaCl_2$ and estimated by distillation in the presence of MgO and Devarda's alloy, respectively. Available P forms (Olsen P) was extracted with 0.5 $molL^{-1}$ $NaHCO_3$ and measured by spectroscopy. Organic phosphorus was measured after mineralization by combustion of the sample and subtraction of the mineral phosphorus amounts, which had previously been estimated in the laboratory. The mineral amounts were extracted with 1 mol L^{-1} H_2SO_4 and all forms were measured by spectroscopy. Exchangeable forms of potassium ware extracted with 1 mol L^{-1} CH_3COONH_4 and measured by flame Photometer (Essex, UK).

Available forms of Mn, Zn, and Cu were extracted with DTPA (diethylene triamine pentaacetic acid 0.005 mol L^{-1} + $CaCl_2$ 0.01 mol L^{-1} + triethanolamine 0.1 mol L^{-1}) and measured by atomic absorption. For the determination of total metals Mn, Cu and Zn, 1 g of wet material, was digested at 350 ^{0}C + 10 ml HNO_3 + 5 ml $HClO_4$. According to the method described by (Allen et al., 1974) and Varian (1989), the samples were analyzed by Atomic Absorption (Spectroscopy Varian Spectra AA 10 plus, Victoria, Australia), with the use of flame and air-acetylene mixture.

Statistical analyses were performed by the use of statistical program MINITAB (Ryan et al., 2005); data represent average means and SE deviation.

3. Results and discussions

The incorporation of the mixture of water soluble and solid olive mill waste in soil, results in increase of soil organic matter, total-N, exchangeable – K (Table 1).

The electrical conductivity of the soil extract, (1 part soil: 5 parts water), varied relatively at low levels, throughout the whole duration of the cultivation. The electrical conductivity of the soil, found 0.29 dSm^{-1} at the start of cultivation, it increased after the 9th day from the initiation of the culture because of adding a controlled amount of fertilizer. Then electrical conductivity showed an increasing trend, until the 30th day at the value of 0.64 dSm^{-1}, and declining after at lower values until the end of cultivation. At the end of the growing period, electrical conductivity was 0.27 dSm^{-1}.

Despite the waste incorporation in soil, with important corresponding amounts in organic matter of 2,56 tons /ha, at the end of the cultivation the percentage of organic matter reverted to baseline levels; it means that during the cultivation period the soil organic matter decreased about 50% (Table 1).

Table 4, shows the balance of available inorganic elements in the soil, for soil depth of 20 cm, during growing period. The content in soil available N, P, K elements at the end of cultivation period, is due to residual fertilizers and to waste added. The decomposition of waste organic matter added, induced liberation of minerals, contributing to soil enrichment by mineral nutritional elements.

It should also be pointed, that the total concentrations of metals: Na, K, Cu, Zn, Mn, Mg, Ni, Fe, Cd, Cr and Pb at end of the crop, did not show any significant increase, compared with the initial concentrations of the respective metals in the soil, before the application of olive oil mill waste.

Finally, the total production of greenhouse Spinach was 2.5 kg/m^2 and the total production of endive was 1.8 kg/m^2.

4. Conclusions

The mineral base dressing in greenhouse crops of spinach and endive, was replaced by a mixture of solid and water soluble products of olive mill waste, in the ratio of 1solid:3 liquid; the rate applied, of 21 tons of water soluble and 7 tons of solid waste per hectare, corresponds to 17.34 kg of N, 22.63 kg of P_2O_5 and 98.7 kg of K_2O per hectare; the needing of plants were satisfied by the residual fertilizers from previous crops, by profiting the organic matter decomposition liberating minerals, and with complementary addition via irrigation, 23 kg of N, 10 kg of P_2O_5 and 48 kg of K_2O per Ha. The adoption of that fertilization plan, has been monitored by EC systematic measurements, ensuring the normal plant development, and maintaining in low levels the soil salinity.

That study, showed that the incorporation of the mixture of olive mill wastes in soil in the ratio (1solid waste: 3water soluble waste), is a good amendment for greenhouse soil, with vegetable crops; at the end of the growing period, soil salinity was maintained at low levels, no increase of heavy metals was observed, and the crop returned satisfactory yield. Furthermore, the recycling of that waste on crops constitutes a very important solution for environment protection.

Table 1. Chemical properties of the soil, before and after crop development

Property	Soil before the adding of waste	Soil after the adding of waste but before the seeding	Soil after the end of the crop period
Texture	Sandy Loam		
Organic matter (%)	1.14 ± 0.02	2.06 ± 0.03	1.06 ± 0.02
pH (1part : 5parts H_2O)	7.32	7.65	7.96
$CaCO_3$ (%)	5.96 ± 0.6	6.62 ± 0.7	7.5 ± 0.7
Electrical conductivity, extract (1part soil:5parts H_2O) dS m^{-1}	0.72 ± 0.04	0.29 ± 0.01	0.566 ± 0.02
CEC (cmol kg^{-1})	31.2 ± 2.2	31.9 ± 2.2	32.1 ± 2.0
Exchangeable-Na (mg kg^{-1})	310.5 ± 17	171.4 ± 9.1	163.3 ± 7.8
N -total (g kg^{-1})	1.05 ± 0.3	1.87 ± 0.6	1.40 ± 0.4
N-NH_4^+ (mg kg^{-1})	36.9 ± 4.2	20.5 ± 3.2	24.3 ± 3.5
N-NO_3^- (mg kg^{-1})	247.8 ± 33	149.8 ± 16	74.9 ± 7.1
P -Olsen (mg kg^{-1})	22.2 ± 4.1	14.93 ± 3.8	14.50 ± 3.5
K-Total (g kg^{-1})	9.85 ± 0.2	9.85 ± 0.3	8.81 ± 0.2
Exchangeable-K (mg kg^{-1})	341.2 ± 3.5	393.9 ± 3.7	374.4 ± 3.7
Mg-Total (mg kg^{-1})	194.3 ± 7.3	194.4 ± 8.2	194.3 ± 7.7
Na-Total (g kg^{-1})	1.77 ± 0.2	1.49 ± 0.1	1.59 ± 0.2
Cu –Total (mg kg^{-1})	27.8 ± 2.4	28.6 ± 2.6	27.75 ± 2.5
Cu –DTPA (mg kg^{-1})	1.76 ± 0.05	1.79 ± 0.05	1.55 ± 0.04
Zn -Total (mg kg^{-1})	71.5 ± 4.8	69 ± 3.9	64.3 ± 3.3
Zn –DTPA (mg kg^{-1})	1.45 ± 0.04	0.99 ± 0.02	1.28 ± 0.03
Mn -Total (mg kg^{-1})	396.9 ± 24	457.8 ± 29	378 ± 22
Mn -DTPA (mg kg^{-1})	3.26 ± 0.7	2.20 ± 0.3	1.29 ± 0.1
Fe-Total (g kg^{-1})	14.18 ± 0.3	14.19 ± 0.3	14.22 ± 0.2
Ni- Total (mg kg^{-1})	148.6 ± 11	174.2 ± 13	144.1 ± 12
Cd- Total (mg kg^{-1})	<0.02	<0.02	<0.02
Pb- Total (mg kg^{-1})	<0.02	<0.02	<0.02
Cr- Total (mg kg^{-1})	<0.06	<0.06	<0.06

Table 2. Chemical properties of the solid olive mill waste and water soluble waste

Property	Olive mill waste	
	solid olive mill waste (wet basis)	Water soluble olive mill waste (wet basis)
Organic matter (%)	18.5 ± 0.9	6±0.2
pH	4.90 (1 part waste + 5 parts H_2O)	5.10 (raw waste)
Electrical conductivity (dS m^{-1})	0.91 ± 0.02 (1 part waste + 5 parts H_2O) water extract	1.84 ± 0.03 (raw waste)
N -total (g kg^{-1})	0.82 ± 0.04	0.55±0.03
P-organic (mg kg^{-1})	283 ± 30	74±4.1
P-inorganic (mg kg^{-1})	200 ± 23	234±25
K-Total (mg kg^{-1})	777 ± 39	3900± 150
Mg -Total (mg kg^{-1})	342 ± 15	199 ± 9.4
Na-Total (mg kg^{-1})	628 ± 37	71.6 ± 2.2
Cu -Total (mg kg^{-1})	21.9 ± 1.2	13.7 ± 0.9
Zn -Total (mg kg^{-1})	20.9 ± 1.3	6.2 ± 0.8
Mn –Total (mg kg^{-1})	13.6 ± 0.6	6.8 ± 0.5

Table 3. Foliar analysis of plants (dry matter %)

Crop	N (%)	P (%)	K (%)
spinach	5.10	0.41	5,95
endive	3,05	0.29	3.60

Table 4. Balance of available mineral nutrient elements in greenhouse soil at depth of 20 cm

	Mineral-N	Available-P_2O_5	Available-K_2O	Soil electrical conductivity dS m^{-1}
	(kg/ha)			
(*)Start of growing period	408.7	82.1	1139.3	0.290
top dressing	23	10	48	
end of crop period	238.1	79.7	1082.9	0.270

(*) soil sample after waste application

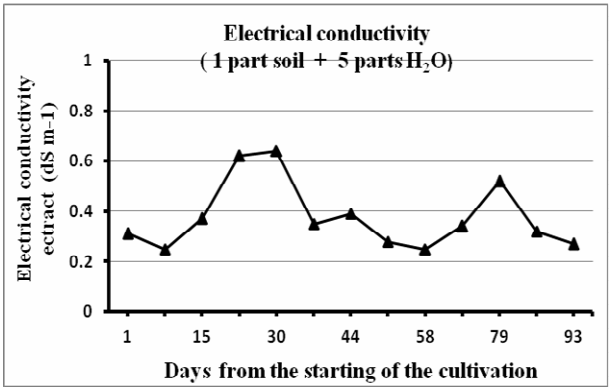

Figure 1. Soil electrical conductivity evolution, during cultivation period

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APPLICATION AND RESEARCH OF SIX SIGMA MANAGEMENT METHOD IN HOTEL FOOD SAFETY CONTROL

Yan Li

Jiangsu Food & Pharmaceutical Science College, Huaian, Jiangsu, 223003, China
liyanjs@163.com

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ABSTRACT
With the social progress and the improvement of people's living level, people have more and more opportunities to eat out and require more on the hotel food. Six sigma management is a set of systematic business improvement system. This paper analyzes the feasibility of six sigma management in hotel food safety control. The basic theory of six sigma management is described. The application of six sigma management in hotel food safety control is discussed. Its introduction in hotel food safety control aims to improve food quality and meet the demand of customers, thus to provides comments and suggestions for the establishment of safe and reliable food safety system.

1. Introduction

Modern hotel is the open enterprise, which can provide accommodation, catering, social contact and entertainment for guests and public. Preventing food poisoning is crucial in hotel food management. General office of the Ministry of Health pointed out in the notification on 2013 national food poisoning that, there were 152 cases of food poisoning reports, 5559 poisoning people and 109 deaths across the country. Among them, family reports accounts for 53.3% with 28.12% poisoning people while the catering service corporations that only accounts for 14.47% is reported to have 21.71% of poisoning people. It illustrates that food safety is very important in catering service corporations and the reconstruction of scientific and high-quality management is the important approach. The six sigma management is characterized by sincere concerns for customs, active management and borderless cooperation and can solve a series of problems. It aims to improve service quality, emphasize process management and continuous improvement, thus to provide strategic guidance for hotel food safety.

2. The basic theory of six sigma management

2.1 The concept of six sigma

Statistically, six sigma (6σ) refers to 6 times of standard deviation. Standard deviation refers to the departure degree of quality from target. The smaller the standard deviation is, the smaller the data dispersibility is. The core concept of six sigma is customer orientation, data motivation, continuous improvement and pursuit of excellence (Zhen, 2013). The solution is determined by brain storming, creative thinking method, optimal practice method, and flow chart. It has made a considerable achievement in manufacture industry. It is gradually applied in service industry and brings good benefits for the service industry (Yining, 2011).

In the perspective of management, six sigma is not only the objective of quality management but also the concept and method for management. It reduces waste and resource

loss through designing and monitoring daily business, emphasizes describing process and guiding decision by data and fact, proposes creative solution, stresses facing to flow and reduces risks by minimum flow defect, thus to increase profit and satisfy customers (Longze, 2013; Weidong, 2012).

2.2 Method of six sigma

The major implementation pattern of six sigma is DMAIC (Guohong et al., 2010) which can be divided into five stages: definition, measurement, analysis, improvement and control. Definition needs to confirm the demand of customer, as well as the key quality characteristics of flow and project needed to be improved; measurement needs to confirm flow index, find out difference, and reconstruct the management of current standard measurement flow; analysis needs to confirm the key impact factor of process through various approaches; improvement need to find the optimal scheme and minimize the defect and fluctuation in improvement process; control is to control sequencing of result, that is, pursue for continuous improvement based on the current result. DMAIC is a closed-loop cycle. Every procedure is dispensable in the process of project improvement. Second, DMAIC is also an open spiraling ring. After every quality improvement, improvement with high quality level starts. It is an upward state that cycles and improves constantly (Huming, 2010).

3. The application of six sigma in hotel food safety control

The demonstration is performed according to the implementation pattern of six sigma-DAMIC. In demonstration process, data about hotel food safe quality is widely collected and investigation and interview are also conducted, thus to ensure facticity and effectiveness.

3.1. D- definition stage

Select six sigma projects for hotel food safety. Hotel food safety project is to improve the deficiency by six sigma method in hotel food safety control. So far, it is found that, the quality of hotel food safety mainly reflects on supplier management, sanitation management, cold chain management, hotel food safety system and staff quality. The data is made further analysis using computer hierarchical analysis software after obtaining data. The judgment matrix and total ordering of the improvement project for the specific problem are shown in Tables1 and 2. It can be seen that in the food safety indexes, supplier weight is the maximum. Hotels regard supplier management as the improvement project of food safety management. The key quality characteristics refers to clear the special requirements of customer and then to set significant assessment standard. The improvement project of hotel food safety control is supplier management. The key quality characteristics of suppliers are expanded as shown in Figure 1.

3.2 M-measurement stage

Measurement is the first step of any improvement (Wu, 2012). The task of this stage is mainly to collect and measure the indexes of improvement project of hotel food safety management, in order to better analyze and obtain management level with flow as well as set rational improvement target. This paper organized an investigation on the status of hotel food supplier. The object of investigation is the managers and staffs from hotel food department. The content is to measure the indexes of supplier management system. The standard of measurement is dissatisfaction, relatively dissatisfaction, relatively satisfaction and satisfaction.

The qualified baseline of hotel supplier management is that the managers and staffs feel satisfactory or relatively satisfactory. Otherwise, it is judged as unqualified or defective. Among them, the defect opportunity of hotel reflects on the satisfaction degree of staff when provided services by supplier, including common, relatively dissatisfaction and dissatisfaction. Defects per opportunity (DPO) refer to the rate of defects appearance in every opportunity and are the proportion of

defects accounting for all opportunities. They are calculated according to the Equation 1.

$$DPO = \frac{\text{Defect number}}{\text{Number of people investigated x the deffect opportunity felt by every investigator}} \quad (1)$$

$$\text{Level improvement rate} = \frac{\text{Improvement target}}{\text{Current level}} \quad (2)$$

$$\text{Absolute weight} = \text{importance degree x level improvement rate x correction coefficient } \alpha_1 \quad (3)$$

$$\text{Absolute weight} = \frac{\text{Absolute weight}}{\text{Sum of absolute weight of quality characteristics}} 100 \quad (4)$$

$$TR_j = \sum_{i=1}^{n} RCR_i x R_{ij} (i = 1,2..n; j = 1,2..m) \quad (5)$$

Table 1. Judgment matrix of six sigma improvement project

Six sigma improvement project	Food safety system management	Sanitation management	Staff quality	Supplier management	Cold chain management
Food safety system management	1	2	2	0.5	0.5
Sanitation management	0.5	1	2	1/4	1/3
Staff quality	1/3	0.5	1	2	1/4
Supplier management	2	4	5	1	2
Cold chain management	2	3	3	0.5	1

Table 2. Hierarchy total sequencing result of six sigma improvement projects

Index name	Supplier management	Cold chain management	Food safety system management	Sanitation management	Staff quality
Index weight ordering	0.3920	0.2682	0.1769	0.0983	0.0626

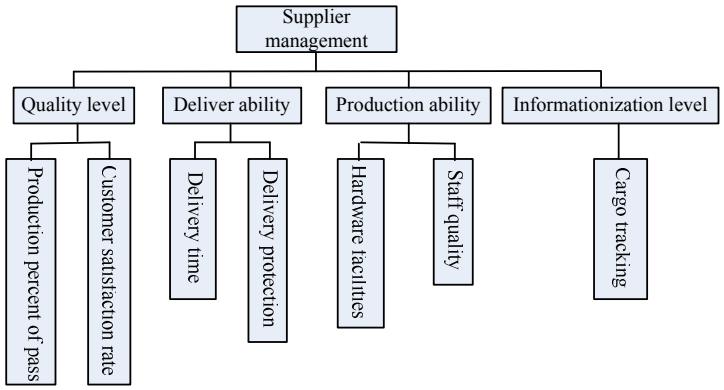

Figure 1. Expansion of key quality characteristics of supplier management

Table 3. Expansion of quality function

Quality characteristics	Reason				Quality planning						
	Commodity inspection	Management mechanism	Supplier quality	Informationization level	Importance degree	Current level	Improvement target	Level improvement rate	Correction coefficient αi	Absolute weight	Relative weight %
Product of percent of pass	2	2	3	0	5	4	5	1.25	1.5	7.5	15.7
Customer satisfaction rate	3	1	2	2	4	3	4	1.33	1.2	6.4	13.37
Delivery time	0	2	2	0	4	3	4	1.33	1.2	8	16.74
Delivery protection	0	2	2	0	4	3	4	1.33	1.2	6.4	13.38
Hardware facilities	0	1	3	0	4	4	5	1.25	1.2	7.5	15.7
Staff quality	0	1	2	0	4	3	4	1.33	1.2	8	16.74
Goods tracking	0	0	1	3	3	3	4	1.33	1	4	8.37
Importance of adopted measureme	71.51	137.45	196.27	51.85	——						

The investigation indicates that, six sigma level of hotel food supplier management, so far, is 2.23σ, illustrating that the hotel food supplier management has big improvement space.

3.3. A-analysis stage

This stage is mainly to analyze the key reason of hotel food safety control affecting supplier management and to provide basis for formulating scheme in the next stage. In analysis stage, four factors with large influence on supplier management are concluded by brain storming: product quality inspection, hotel management system, and supplier quality and hotel informationization level. Reason is analyzed in the perspective of quality function and the problem analysis figure is drew (Table 3). The process is as follows:

The characteristics of hotel food supplier management quality are expanded (Table 3). Matrix intersection refers to the relationship degree between influence factor and quality characteristics. "0, 1, 2, 3" stand for no relationship, weak relationship, medium relationship and strong relationship. The importance of quality characteristics of food supplier needed to improve can be divided into unimportance, relatively unimportance, common, relatively importance and importance. The satisfaction degree of specific quality characteristics of supermarket are 1-5 level, representing the current level of expanded quality characteristics. The improvement target is set as improving one grade on the previous level. Its correction coefficient is assigned. 1.5 means important characteristic, 1.2 means relatively important characteristics and 1.0 means common characteristics. The calculation formula is indicated in Eq. 2-4. Analysis of quality characteristics of hotel food supplier management: suppose RCRi as the relative important degree of no.i quality characteristics, Rij as the relationship degree between no.i quality characteristics of no.j adopted measurement, and TRj as the importance of no.j measurement, then the Eq. 5 comes true. It can be seen from Table 3 that, the importance degree of the measurement adopted on supplier management is selection and assessment of supplier, perfection of management system, strengthening of commodity inspection and establishment and perfection of information system.

3.4. I-improvement stage

The main task of this stage is to summarize the first three stages of work and to propose corresponding measurement and scheme for the influence factors of hotel food safety supply.

Strengthen the assessment and selection of supplier. The assessment and selection of supplier are inseparable. A good supplier has excellent score on assessment. The selection of supplier by hotel reflects on high-quality product and service and high work efficiency. Therefore, hotel should combine their own advantages and select the best supplier to offer high-quality product and service.

Hotel management mechanism on supplier mainly contains the monitoring mechanism and incentive mechanism. Hotel should monitor the supplier, strengthen finished product inspection and purchase record, and organize hotel managers to guide supplier timelessly. Meanwhile, the hotel should encourage supplier with good reputation such as implementing reputation incentive or offering reverse price discount for supplier and eliminate supplier with bad quality.

Strengthen the inspection of hotel on food. In the inspection process, hotel should establish reliable food safety quality security system, implement food hygiene responsibility in every step and monitor corresponding system index, thus to achieve the requirement of hotel on food.

Establish and perfect information system. Hotel system is a kind of service business. Its purpose is to satisfy customer to the largest extent, master the information of supplier and circulating state of transit warehouse and transportation steps comprehensively and accurately, which is the premise for

guaranteeing both quality and quantity for customer. In addition, hotels need to release order if necessary to deal with emergency. All these things need the support from information system.

3.5. B-control stage

This stage is mainly to control improvement effect and consolidate improvement achievement. Its purpose is to continue the improved achievement and avoid failure. Six sigma group of hotel formulate a set of control plan based on the improved achievement and new program, mainly including: (1) purchasing director is responsible for master every step in the purchasing process; purchasing staff is responsible for his supplier; (2) formulate business flow. Hotel staff should work following procedure; the work should have responsibility system; report once find the problem; complete food safety management work; (3) when accident happen, such as metamorphic and overdue food, hotel should contact with supplier to avoid food safety event; (4) keep strictly the rules for reward and punishment; reward the supplier with good performance, otherwise, implement punishment.

So far, one round of project improvement is basically completed. Through that, the supplier of hotel improves greatly as well as the management system of hotel and the satisfaction degree of customer also increases. However, the improvement of six sigma project is everlasting. The six sigma group in hotel should formulate continuous improvement plan for supplier management, measure the supply process of supplier and the food demand of hotel timelessly, analyze the relationship between them, find out the difference and ensure the continuous effect of achievement. Meanwhile, six sigma management should be promoted into other aspects, thus to improve food safety management, meet the demand of customer and enhance the core competition of hotel.

4. Conclusions

Six sigma theories is a kind of management method to improve flow and enhance quality and structure of product. Its main concept is to pursue zero defect production, keep away product responsibility risk, lower cost, improve competition rate and customer satisfaction, thus to realize its strategic objective (Yihua and Zhenzhen, 2011). Six sigma is applied in hotel food safety control to analyze it, find out the main problem, propose improvement scheme and precede application practice. The result indicates that, through the improvement by six sigma management method, the food safety control of hotel improves greatly, thus enhance the service capacity and competitiveness of hotel. Meanwhile, the implementation of six sigma management requires the participator possess certain knowledge. Therefore, hotel should strengthen the knowledge training for staff, thus to better promote the application of this management system.

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OPTIMIZATION CONDITIONS FOR ANTHOCYANINS EXTRACTION FROM RED CABBAGE USING RESPONSE SURFACE METHODOLOGY

Yu Zou*, Chenghui Liu

College of Life Science, Dalian Nationalities University, Dalian, Liaoning, 116600, China
** yuzou126@126.com*

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ABSTRACT
Anthocyanin is a natural pigment with great development potential as a healthful food colorant. In this paper, extraction conditions of anthocyanins from red cabbage were investigated. Liquid-solid ratio, temperature and duration were chosen to optimize extraction conditions using response surface methodology (RSM). The Box-Behnken experimental results showed the optimum extraction conditions as follows: a liquid-solid ratio of 27.35 mL/g, a temperature of 29.94 °C and duration of 39.47 min. Under these conditions, the maximal anthocyanins yield was 128.78 mg/100 g. The results of this study showed that red cabbage could be used as a good source of the new-type natural anthocyanins.

1. Introduction

Frequent discovery of synthetic pigments' harmful effects on human health has led to public interest in natural pigments as alternatives in food industry, resulting in increasing study on natural colorants. Anthocyanins widely exist in the roots, stems and leaves as well as flowers and fruits of the high plants (Fan et al., 2008). Anthocyanins derived from various biological, such as purple sweet potato (Fan et al., 2008), purple corn (Yang et al., 2008), black rice (Boo et al., 2012), yellow paprika (Boo et al., 2012), grape peel (Boo et al., 2012) and mulberry (Boo et al., 2012) do not have any toxic, teratogenic and mutagenic properties, even at high doses of these compounds. In food products, the colors of anthocyanins can increase the esthetic value of food. Depending on pH of food products, anthocyanins are the source of red, orange, blue and purple colour, which usually makes foods more attractive for consumption (Wiczkowski et al., 2014). As is shown in previous studies, anthocyanins has a number of healthful functions, such as antioxidation (Boo et al., 2012; Wiczkowski et al., 2014), anti-inflammatory (Giusti and Wrolstad, 2003) and anti-neoplastic activity (Xu et al., 2010). These functions promise natural anthocyanins with great development potential as a healthful food colorant.

Red cabbage (*Brassica oleracea* L. var. *capitata f. rubra*) belongs to the family of Brassicaceae, which is a native vegetable of the Mediterranean region and southwestern Europe (Arapitsas et al., 2008). Red cabbage contains a high level of anthocyanins, compared to white and yellow ones. At present, the extraction of anthocyanins from red cabbage reported is focused on analyzing extraction (Walkowiak-Tomczak and Czapski, 2007; Li et al., 2014; Xu et al., 2014; Volden et al., 2008). However, there is little information available on optimizing extraction conditions of anthocyanins using an affective statistical technique. This paper was to develop an economical and efficient extraction of anthocyanins from red cabbage. Response surface methodology (RSM) was employed to optimize extraction conditions (liquid-solid

ratio, temperature and duration) in order to obtain the maximal anthocyanins yield.

2. Materials and methods

2.1. Materials

Fresh red cabbage was purchased from a local wholesaling market in Dalian in July 2014. Every 1 kg red cabbage shred in a polyethylene bag was stored at -18 °C for further extraction experiments after being frozen at -40 °C for 48 h. Prior to extraction, frozen samples for experiments were crushed in a pulper (Tianjing, FSH-2, Jintan, China) for 1 min with 10 s intervals to avoid samples to be heated.

2.2. Extraction of anthocyanins

The extraction process of anthocyanins was carried out_according to the method of Fan et al. (2008) with proper modification. Red cabbage sample were put into a 50 mL conical flask, then added in acid-ethanol (HCl, 1.5 mol/L) with different liquid-solid ratio (20-30) and put in thermostatic water bath (Zhongxin, DSK-24, Jiaxin, China) at selected temperatures (25-35 °C) for various duration periods of time (35-45 min), then, centrifuged at 4000 rpm for 15 min. The supernatant was collected and transferred into 50 mL volumetric flask for the determination of anthocyanins yield.

2.3. Experimental design

The Box-Behnken experimental design with three factors and three levels was employed to optimize the extraction conditions in order to obtain the highest anthocyanins yield. Liquid-solid ratio (*A*) , temperature (*B*) and duration (*C*) were chosen as independent variables in this design. *A* (20, 25 and 30 mL/g), *B* (25, 30 and 35 °C) and *C* (35, 40 and 45 min) were determined as critical levels with significant effect on anthocyanins extraction. The complete design consisted of seventeen combinations including five replicates of the center point (Table 1).

The experimental results were analysed by quadratic stepwise regression to fit the second-order equation (1):

$$Y=\beta_0+\sum_{i=1}^{3}B_iX_i+\sum_{i=1}^{3}B_{ii}X_i^2+\sum_{i=1}^{3}B_{ij}X_iX_j \qquad (1)$$

where: Y standed for anthocyanins yield, X_1, X_2, X_3 for independent variables, β_0 for the model intercept and B_i, B_{ii}, B_{ij} for regression coefficients of variables for intercept, linear, quadratic and interaction terms, respectively. The software Design-Expert 7.0.0 Trial (State-Ease Inc., Minneapolis, USA) was used to obtain the coefficients of the quadratic polynomial model.

2.4. Determination of anthocyanins

The concentration of anthocyanins was determined applying the Lambert-Beer law (Francis, 1989). The spectra recorded in a diode array spectrophotometer (UNIC, UV-2802, USA) were measured at 25 °C and 530 nm against the solvent using 1-cm quartz cells. The anthocyanins yield (mg/100 g) was calculated using the following Equation 2:

$$\text{Anthocyanins yield} = A_{530}\times\text{dilution factor}/98.2 \qquad (2)$$

where: A_{530} was the absorbance in the diluted sample. The factor 98.2 was the molar absorptive value for the acid-ethanol solvent and it referred to the absorption of a mixture of cranberry anthocyanins in acid-ethanol, measured in a 1-cm cell at 530 nm, at a concentration of 1% (w/v).

2.5. Statistical analysis

The experimental results obtained were expressed as means ± SD of triplicates. Statistical analysis was performed using Fisher's *F*-test. $p<0.05$ was regarded as significant and $p<0.01$ as very significant.

3. Results and discussions

3.1. Analysis of Box-Behnken experiment

The extraction conditions including liquid-solid ratio, temperature and duration as independent variables were further optimized

for the maximum anthocyanins yield. The Box-Behnken design and the corresponding response values were shown in Table 2.

By using the Design Expert software, a second-order polynomial model describing the correlation between anthocyanins yield and the three variables in this study was obtained in Equation 3 below:

$$Y = -1457.85 + 7.89A + 83.56B + 11.44C - 0.13A^2 - 1.32B^2 - 0.08C^2 + 0.02AB - 0.04AC - 0.13BC \quad (2)$$

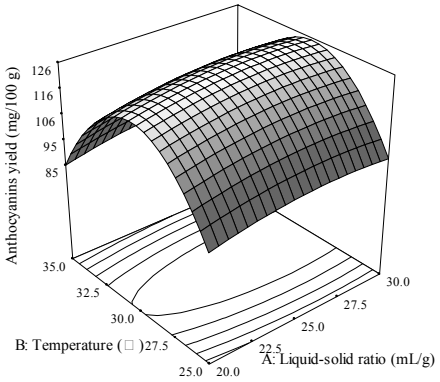

Figure 1. Response surface plot showing the effect of liquid-solid ratio and temperature on anthocyanins extraction from red cabbage (constant contact time at 40 min)

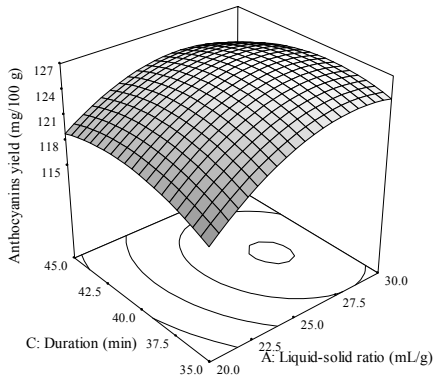

Figure 2. Response surface plot showing the effect of liquid-solid ratio and duration on anthocyanins extraction from red cabbage (constant temperature at 30 °C).

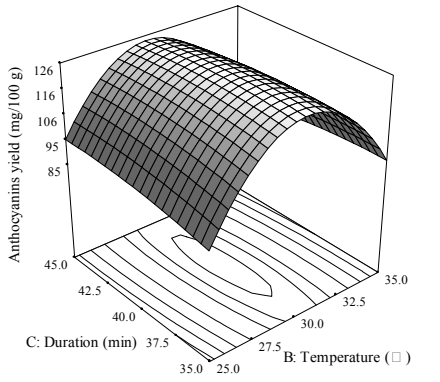

Figure 3. Response surface plot showing the effect of temperature and duration on anthocyanins extraction from red cabbage (constant liquid-solid ratio at 25 mL : 1g).

Table 1. Independent variables and their coded and actual values used for optimization

Independent variable	Symbol	Code levels		
		-1	0	+1
Liquid-solid ratio (mL/g)	*A*	20	25	30
Temperature (°C)	*B*	25	30	35
Duration (min)	*C*	35	40	45

Table 2. The Box–Behnken design and experiment data for anthocyanins extraction

Run	*A*	*B*	*C*	Anthocyanins yield (mg/100 g)	
	liquid-solid ratio (mL/g)	Temperature (℃)	Duration (min)	Observed value	Predicted value
1	0	-1	1	90.75±3.18	92.55
2	0	-1	-1	117.00±3.24	118.85
3	0	1	1	127.50±2.17	125.75
4	-1	1	0	87.70±2.28	93.40
5	0	0	0	124.00±2.70	125.75
6	-1	0	-1	91.50±2.47	91.55
7	0	0	0	127.50±3.32	125.75
8	1	1	0	90.60±1.19	84.90
9	0	0	0	92.75±2.21	88.90
10	1	0	1	126.30±2.98	124.45
11	0	0	0	82.25±1.02	86.10
12	1	0	-1	97.25±1.98	95.45
13	0	0	0	126.30±2.24	122.40
14	-1	-1	0	112.80±1.08	116.70
15	-1	0	1	125.75±1.87	125.75
16	0	1	-1	124.00±2.43	125.75
17	1	-1	0	88.80±1.91	88.75

Table 3. Analysis of variance (ANOVA) of the response surface regression model

Source	Sum of squares	Degree of freedom	Mean squares	*F*-value	*P*-value
Model	4874.63	9	541.63	25.17	0.0002
A	63.85	1	63.85	2.97	0.1286
B	0.01	1	0.01	0.75	0.4137
C	0.02	1	0.02	0.01	0.9883
A^2	41.12	1	41.12	1.91	0.2094
B^2	4578.32	1	4578.32	212.77	< 0.0001
C^2	17.27	1	17.27	0.80	0.4001
AB	1.00	1	1.00	0.05	0.8355
AC	4.41	1	4.41	0.20	0.6645
BC	42.25	1	42.25	1.96	0.2039
Residual	150.63	7	21.52		
Pure Error	12.25	4	3.06		
Corrected Total	5025.25	16			

$R^2 = 0.9700$, adj $R^2 = 0.93$

The statistical significance of equation (3) was checked by *F*-test, and the results of analysis of variance (ANOVA) were shown in Table 3. The model *P*-value of 0.0002 obtained by ANOVA indicated that the model was significant ($p<0.05$). For the model fitted, the coefficient of determination (R^2), which could check the goodness of a model (Sun et al., 2009), was 0.9315 implying that the sample variation of 93.15% for the anthocyanins yield was attributed to the independent variables, and only 6.85% of the total variation could not be explained by the model. These results suggested that the developed model could adequately represent the real relationship among the parameters chosen.

3.2. Effects of liquid-solid ratio, temperature and duration on anthocyanins extraction

As was shown in Table 3, temperature had significant quadratic effect ($p<0.05$) on anthocyanins extraction. However, none of the independent variables (liquid-solid ratio, temperature and duration) interacted significantly ($p>0.05$).

Figure 1 showed the effect of liquid-solid ratio and temperature on anthocyanins extraction from red cabbage at a constant duration of 40 min. At a fixed temperature, the anthocyanins yield increased slightly when liquid-solid ratio was raised. This indicated that the increase in liquid-solid ratio could not further increase the anthocyanins yield. The anthocyanins yield increased when temperature was raised from 20°C to 30°C but rapidly

decreased when temperature continued to be raised. This phenomenon could be explained in terms of colorant degradation. The anthocyanins might degrade due to treatment under high temperature condition (Tiwari et al., 2009). Therefore, the optimum temperature should be about 30 °C in the present work.

Figure 2 showed the effect of liquid-solid ratio and duration on anthocyanins extraction from red cabbage at a constant temperature of 30 °C. At a fixed duration, the increase in liquid-solid ratio led to a gradual increase of anthocyanins yield but it declined later. When liquid-solid ratio was set, the anthocyanins yield increased rapidly when duration was extended, but it reached a constant value when duration was above 40 min. The anthocyanins yield could not increase when duration continued to be extended. This result was similar to those previously reported by Rodrigues et al. [(2008). These findings make the whole process of anthocyanins extraction economically more feasible and efficient in the potential application in food industry.

Figure 3 showed the effect of temperature and duration on anthocyanins extraction from red cabbage at a constant liquid-solid ratio of 25 mL/g. At a fixed duration, anthocyanins yield increased rapidly when temperature was raised, but it gradually decreased when the temperature was above 30 °C. This implied that the anthocyanins yield was significantly influenced by temperature. At a fixed temperature, the variety of anthocyanins yield was slight when the duration increased, especially when the duration exceeded 40 min.

3.3. Optimization of extraction conditions and Verification of the model

According to the RSM test results, the optimal extraction conditions to obtain the highest anthocyanins yield were determined as follows: a liquid-solid ratio of 27.35 mL/g, the temperature of 29.94 °C and the duration of 39.47 min. The anthocyanins yield was 128.78±2.24 mg/100 g, and this value was not significantly different (p>0.05) from the predicted value of 128.42 mg/100 g. These data proved that the model designed in this study was valid.

4. Conclusions

In this study, liquid-solid ratio, temperature and duration were chosen to optimize anthocyanins extraction by a three variable, three level Box-Behnken experiment design. The combination of liquid-solid ratio (27.35 mL/g), temperature (29.94 °C) and duration (39.47 min) was determined to obtain the highest anthocyanins yield (128.78 mg/100 g). The experimental results showed that red cabbage could be used as a good source of the new-type natural anthocyanins.

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EFFECT OF PRE-DEHULLING TREATMENTS ON CHEMICAL COMPOSITION, FUNCTIONAL AND PASTING PROPERTIES OF WHOLE OAT FLOUR

Jaspreet Kaur[1]*, Amarjeet Kaur[1], Preeti Ahluwalia[1]

[1]*Department of Food Science and Technology, Punjab Agricultural University, Ludhiana, Punjab, India,*
**jasciphet@gmail.com*

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ABSTRACT
Oat grains were subjected to treatments such as soaking in water, soaking in 0.1% sodium hydroxide and hydrothermal treatment (at 35±2% moisture and steaming at 1.05 kg/m2; for 5, 10 and 15 min). Treated grains were hand dehulled and groats obtained were dried to 11±0.5% moisture. Flour obtained from each treatment was analyzed for chemical composition, functional and pasting properties and compared with the untreated oat flour. As compared to control, hydrothermally treated samples had significantly ($P \leq 0.05$) lower contents of NDF (neutral detergent fibre), ADF (acid detergent fibre), ADL (acid detergent lignin), hemicellulose and cellulose. The resultant flours had improved product making properties. Further, hydrothermal treatment of grains for 15 min significantly ($P \leq 0.05$) improved physical and functional properties such as lightness, bulk density, WAI (water absorption index), WSI (water solubility index) and OAC (oil absorption capacity) of flour. Compared to control, treated flour samples had significantly ($P \leq 0.05$) low peak and final viscosities.

1. Introduction

Oats (*Avena sativa*) are acknowledged worldwide as excellent functional food. High contents of carbohydrates, protein, fat, minerals, vitamins, dietary fibre and phytochemicals such as avenanthramides and β-glucan make oats ideal functional ingredients in bakery and pasta products (Mariotti et al., 2006). The consumption of fibre-rich foods such as oats, prevents alimentary canal disorders, reduces blood glucose and cholesterol level and lowers the risk of colon cancer (Gambus, 2011). Traditionally, in developing countries such as India, oats have not been used for human food as much as other cereals. Oats are grown sparsely in India and their use is mainly restricted to animal feed and fodder. Almost 1.0 lakh hectares of land is under oat cultivation in India (ICAR, 2006). Technology for processing locally available varieties of oats into flour has also not been developed.

During processing oat grains into flour, dehulling is an important yet difficult unit operation. In mechanical dehulling, some amount of hull remains attached to groats (Doehlert et al., 2010). Further, separation of undehulled grains from groats is difficult and labour-intensive and hull fractions may be retained in flour. Hull is rich in indigestible fibre constituents which include remnants of plant cell wall material that are not hydrolyzed by enzymes of the alimentary canal. It is a complex entity that mainly includes cellulose, hemicellulose and lignin (Baker, 1977). Hence, presence of hull affects composition of flour. Further, fibre may lower acceptability of oat flour and products.

Pre-dehulling treatments may help loosen hull for easier separation from kernel. Soaking and hydrothermal treatments have been found effective in improving dehulling efficiency of several food grains such as pigeon pea (Tiwari et al., 2008) and canola (Mohamadzadeh et al., 2009). During commercial production of oat products hydrothermal treatment of groats is done after dehulling. High fat content (3-8%) and high lipase activity lower shelf-life of oat products (Ekstrand et al., 1992) and necessitate inactivation treatments. Hydrothermal treatments of oat grains have also been found effective in altering functional properties and improving product making quality of oat flour (Zhang et al., 1998; Gujral et al., 2013). Functional properties of oat flour and isolated oat protein have been evaluated and reported in literature (Mirmoghtadaie et al., 2009; Mohamed et al., 2009).

Since flour properties are important for product development and are dependent on pre-treatments, this study was planned to study the effect of pre-dehulling treatments on chemical composition, techno-functional and pasting properties of whole oat flour.

2.Material and methods

2.1. Material and chemicals

Oat grains (variety OL-9), grown in the year 2012, were procured from Punjab Agricultural University, Ludhiana, Punjab, India. A combination of cleaning techniques was used for separation of husk, straw, stones etc. from the grains. Physical properties and moisture content of the grains were observed. Cleaned and dried grains were stored in air tight plastic containers at 10±2 °C till further use. All chemicals for chemical analyses were of analytical grade and procured from Central Drug House, New Delhi, India.

2.2. Pre-dehulling treatments

Oat grains were divided into six sets of 100 g each and five of them were given separate treatments as shown in Table 1. During each treatment, grains were soaked overnight (14 h) to 35±2% moisture.

2.3. Dehulling

After treatments, hull was manually separated from the grain samples. Groats resulting from AS treatment (alkali soaked; overnight in 0.1% sodium hydroxide) were washed in tap water to remove any traces of sodium hydroxide. Thereafter, each set of groats was dried in tray drier (50±5 °C) to 11±0.5% moisture. For control, oat grains were dehulled with Impact Huller (Lab Impact-II, Creative India, Mohali, Punjab, India) without any previous treatment. Dehulled grains were separated from husk with a laboratory aspirator and undehulled grains were manually separated from the groats.

2.4. Preparation of flour

Groats obtained from the samples were pulverized in cyclone mill to obtain whole flour of 60 BSS particle size and packed in polyethylene bags and kept at 10±2 °C till further use.

2.5. Chemical analysis

Moisture, crude protein, crude fat, ash and crude fibre contents of oat grains and flour samples were determined by AACC (AACC, 2000) methods. Carbohydrate content was calculated by subtracting sum of moisture, crude protein, crude fat and ash content from 100 (Merrill and Watt, 1973). Fiber composition of grain and flour samples was determined in terms of neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) using method of Van Soest (Van Soest, 1967). As per this method, hot extraction of the samples was done using respective detergent solutions. Hemicellulose content was calculated from difference between NDF and ADF while cellulose content was estimated from the amount of ADF residue that dissolved in 72% sulphuric acid.

2.6. Physical properties

Bulk density of samples was determined as per procedure described by Akpapunam and Markakis (Akpapunam and Markakis, 1981). Grain/flour sample was put into a weighed 5-

mL measuring cylinder up to the mark. It was gently tapped to eliminate air space volume of sample was observed. Mass of the sample was obtained by subtracting mass of cylinder from mass of the cylinder and sample. Bulk density was expressed as ratio of mass to volume of sample.

Colour values in terms of *L*, *a* and *b* were measured using Hunter Lab Scan XE (Hunter Associates Laboratory Inc., Reston, Virginia, USA) (NR-3000; 10°/D65). *L* value represents lightness (0-100), *a* value represents greenness/redness (-/+) and *b* value represents blueness /yellowness (-/+).

2.7. Functional properties

WAI (water absorption index) and WSI (water solubility index) were determined according to method of Anderson et al. (1969). Oat flour (2.5 g) was suspended in 25 ml water at room temperature (15-35 °C) for 30 min, gently stirred during this period, and then centrifuged at 3000 g for 15 min. The supernatants were decanted into an evaporating dish of known weight. WAI was calculated as ratio of the weight gain by gel to dry weight of sample and WSI was calculated as ratio of weight of dry solids in supernatant to 100 g dry weight of sample. OAC (oil absorption capacity) was determined according to method of Lin et al. (1974). Flour (0.5 g) was mixed with 10 ml of refined oil (Sunflower Refined Oil, Markfed brand, India; bulk density 0.895 g/ ml) in pre-weighed centrifuge tube and vortexed for 10 min. The tubes were centrifuged for 25 min at 3000 g. Oil was drained off by inverting for 10 min and centrifuge tubes were weighed. OAC was calculated as volume (ml) of oil held per gram of sample.

2.8. Pasting properties

Rapid Visco-Analyzer (Newport Scientific Pvt. Ltd., Wartiewood, NSW, Australia) was used to determine pasting properties of flour. A suspension of 3.5 g flour (14% moisture, wet basis) in 25 ml of distilled water underwent controlled heating and cooling cycle at constant shear. RVA plot of viscosity (cP) vs. time (s) was used to determine PV (peak viscosity, BDV (breakdown viscosity), setback viscosity (SB) and final viscosity (FV) AACC, 2000).

2.9. Statistical analysis

All statistical procedures were performed using SPSS (version 16.0) SPSS Inc (Chicago, USA). A one-way analysis of variance (ANOVA) was carried out using completely randomized design and the means were compared using Duncan's Multiple Range Test at $P \leq 0.05$. The results are presented as means±S.D. (standard deviation) of triplicate analyses.

3. Results and discussion

3.1. Physico-chemical properties of untreated oat grains and flour

Oat grains (*Avena sativa*) of variety OL-9 had 1000-grain weight of 29.180 g and bulk density 0.479 g/ml. Oat grains (untreated) were dehulled mechanically and groats were milled to obtain whole flour (control). Oat grains and flour were analyzed for crude protein, crude fat, ash and total carbohydrates, crude fibre, NDF, ADF, ADL, hemicellulose and cellulose (Table 2). Oat grains had lower crude protein (11.973%) and crude fat (4.728%) contents than oat flour, on dry weight basis. Results also indicated high levels of hemicellulose (18.603%) in oat grains. Hemicellulose, cellulose and lignin fractions are insoluble and indigestible by humans. Relatively low energy and nutritive value and high content of indigestible fibre necessitate dehulling of oat grains before further processing for human food. However, oat husk (hull) has been found to contain considerable amounts of xylans that may be used for preparation of fibre-enriched foods (FAO, 1998).

3.2. Chemical composition of treated flours

Flour obtained from untreated (control) and treated grains were analyzed for proximate principles and fibre composition. No significant differences, in terms of crude protein and crude fat contents, were observed between control

and treated flour samples (data not given). However significant differences among control and treated flour samples were observed for fibre composition, crude fibre and ash (Table 3). Observed contents of NDF (neutral detergent fibre; cell wall material) and ADF (acid detergent fibre; lignocellulose), for treated samples, were significantly ($P \leq 0.05$) lower than control. This indicated that mechanically dehulled control samples may be carrying some fibre-rich hull fractions that contributed to higher NDF and ADF than treated flour samples. NDF and ADF contents for treated samples ranged 6.906%-13.610% and 1.478%-2.544%, respectively, on dry weight basis. Although AS (alkali soaked) samples had lower hemicellulose content than WS (water soaked), the difference was not significant. It has been reported that hemicellulose exhibits high solubility in alkaline solutions (Cheng et al., 2010). Cellulose content in WS and AS flour samples was significantly ($P \leq 0.05$) lower than for control flour. This may be attributed to leaching of soluble fragments in the soaking medium.

Hence results indicated that pre-dehulling treatments of oat grains were effective in loosening and removal of hull. Lower fibre contents in flour obtained after hydrothermal treatment indicate higher dehulling efficiency of the treatment (Mohamadzadeh et al., 2009). Soaking and steaming treatments cause leaching of gums and pectinaceous material between the seed coat and endosperm. Moreover, differential expansion of the two components results in loosening of hull from groat (Shobana and Malleshi, 2007). Further, heat-treatment may lead to breakage of weak bonds between polysaccharide chains; and glycosidic linkages in the dietary fibre polysaccharides. This may lead to solubilization and decreased content of dietary fibre. Siljeström et al. (1986) reported loss of insoluble dietary fibre in wheat flour after autoclaving, which was attributed to degradation of the arabinoxylans. Such structural alterations are important for the functional and nutritional properties of the product and its palatability.

Crude fibre content of control flour was significantly ($P \leq 0.05$) higher than all the treatments. AS samples had lowest crude fibre content (1.852%). Although ash content of control flour was higher, hydrothermally treated samples had significantly ($P \leq 0.05$) higher contents than other treated flours. Hydrothermal treatments may have caused the minerals to seep in deeper into the endosperm, resulting in higher ash content.

3.3. Physical properties of flour

Observed bulk density for WS samples was significantly ($P \leq 0.05$) lower than control. Activation of certain enzymes during soaking may be responsible for this phenomenon (Elmoneim et al., 2010). Although bulk density for AS samples was lower than control flour, it was higher than WS flour. Hydrothermally treated flour samples had significantly ($P \leq 0.05$) higher bulk density than control. Further, bulk density increased gradually with increase in steaming time of grains. Bulk density values for control flour and H-15 flour were 0.438 g/ml and 0.496 g/ml, respectively. Similar effect has also been observed for maize flour obtained from hydrothermally treated grains (Bolade et al., 2002). High bulk density is an advantage for storage and transportation as greater quantity of flour can be packed in a given volume of container or space.

Colour (Hunter *L*, *a* and *b* values) of flour samples was significantly affected by pre-dehulling treatments of grain (Table 4). *L* value, that indicates lightness, varied significantly among treatments. Control flour had lowest *L* value (81.79). *L* value of hydrothermally treated flour increased gradually with increase in time of steaming and was highest for H-15 samples (84.79). Negative *a* value indicates green colour whereas higher *b* value indicates more yellow colour. Lowest *a* value was observed for AS samples, whereas highest *b* value was exhibited by H-10 samples. Colour values seem to be affected by husk contents as husk imparts colour to flour.

3.4. Functional properties

WAI (water absorption index) is an important functional property that indicates the ability of flour to associate with water. WAI varied significantly among treatments from 1.255 to 2.940 g/ml. Soaking in water caused significant ($P \leq 0.05$) increase in WAI. Activation of enzymes in the grain during soaking may have lead to breakdown of complex polysaccharide and increased WAI.

Alkali soaking of grains also caused increase of WAI, although this increase was less than for water soaked samples. WAI showed a significant increase with the hydrothermal treatment (Table 4). A gradual increase in WAI was observed from 2.431 to 2.940 g/ml with increase in duration of steaming from 5 to 15 min, respectively. Yadav et al. (2012) also reported similar increase in WAI of flour obtained from hydrothermally treated pearl millet grains. WSI (water solubility index) of WS samples was significantly ($P \leq 0.05$) higher than control. Similarly, AS samples exhibited significantly higher WSI than control. During soaking, reaction between oat starch and sodium hydroxide may have lead to increased cold water solubility (Ruan et al., 2012). Hydrothermally treated flour samples exhibited significantly higher WSI which increased with increase in steaming time. Cooking treatments have been associated with increased starch solubility (Jones et al., 2000).

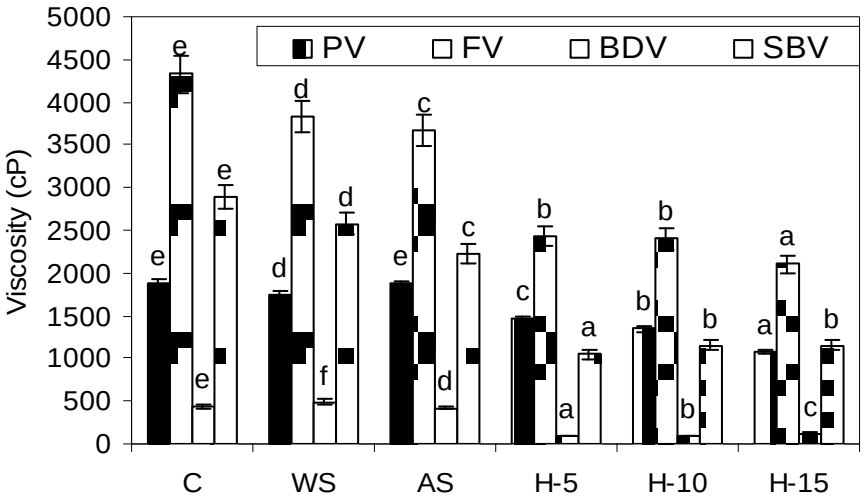

Figure 1. Pasting properties (PV, peak viscosity; FV, final viscosity; BDV, breakdown viscosity; SBV, setback viscosity) for flour of control (C), water soaked (WS), alkali (0.1% NaOH) soaked (AS), hydrothermally treated for 5 min (H-5), hydrothermally treated for 10 min (H-10) and hydrothermally treated for 15 min (H-15) samples (Different letters (a, b, c, d and e) mean significant differences ($P \leq 0.05$) in pasting properties between various samples based upon Duncan's Test)

Table 1. Pre-dehulling treatments given to oat grains

Symbol	Treatment given
C	Control; untreated
WS	Water soaked; soaked overnight in distilled water (25 ± 2°C)
AS	Alkali soaked; soaked overnight in alkali (25 ± 2 °C, 1% sodium hydroxide was used)
H-5	Soaked overnight in distilled water (25 ± 2 °C) and steamed in autoclave at 1.05 kg cm^{-2} for 5 min
H-10	Soaked overnight in distilled water (25 ± 2 °C) and steamed in autoclave at 1.05 kg cm^{-2} for 10 min
H-15	Soaked overnight in distilled water (25 ± 2 °C) and steamed in autoclave at 1.05 kg cm^{-2} for 15 min

Table 2. Proximate composition of untreated oat (OL-9) grains and flour (n=3)

Component	Oat grain	Oat flour
Moisture	9.882 ± 0.122	8.924 ± 0.136
Crude protein	11.973 ± 0.150	16.065 ± 0.043
Crude fat	4.728 ± 0.046	6.177 ± 0.022
Ash	4.098 ± 0.241	1.334 ± 0.01
Total carbohydrates	69.319 ± 0.441	67.5 ± 0.789
Crude fibre	19.107 ± 0.516	4.965 ± 0.455
NDF[A]	31.560 ± 1.027	15.473 ± 1.150
ADF[B]	12.957 ± 0.643	3.075 ± 0.643
ADL[C]	3.435 ± 0.119	1.318 ± 0.021
Hemicellulose	18.603 ± 1.437	12.398 ± 1.101
Cellulose	9.522 ± 0.524	1.757 ± 0.028

All values are expressed in %, dry weight basis (except moisture)
[A] Neutral detergent fibre; [B] Acid detergent fibre; [C] Acid detergent lignin

Table 3. Effect of pre-dehulling treatments on fibre composition (%, dry weight basis) and ash content (%, dry weight basis) of oat flour (n=3)

Treatment	NDF [A]	ADF[B]	ADL[C]	Hemicellulose	Cellulose	Crude fibers	Ash
C	15.473^{e} ± 1.150	3.075^{d} ± 0.643	1.318^{bc} ± 0.021	12.398^{c} ± 1.101	1.757^{c} ± 0.028	4.965^{c} ± 0.455	1.334^{bc} ± 0.01
WS	13.610^{d} ± 0.602	2.397^{bc} ± 0.049	1.505^{c} ± 0.280	11.214^{c} ± 0.952	0.891^{b} ± 0.647	3.619^{b} ± 0.168	1.196^{a} ± 0.067
AS	12.219^{cd} ± 0.944	1.478^{a} ± 0.406	1.285^{b} ± 0.022	10.741^{c} ± 0.982	0.193^{a} ± 0.025	1.852^{a} ± 0.291	1.210^{a} ± 0.050
H-5	11.454^{bc} ± 1.455	2.544^{c} ± 0.315	0.854^{a} ± 0.037	8.910^{b} ± 1.747	1.690^{c} ± 0.352	3.675^{b} ± 0.113	1.236^{ab} ±0.127
H-10	10.020^{b} ± 0.077	2.293^{b} ± 0.268	0.671^{a} ± 0.225	7.728^{b} ± 0.285	1.621^{c} ± 0.434	3.423^{b} ± 0.319	1.429^{cd} ±0.057
H-15	6.907^{a} ± 0.315	1.599^{a} ± 0.012	0.799^{a} ± 0.006	5.308^{a} ± 0.303	0.799^{ab} ± 0.006	2.092^{a} ± 0.297	1.47^{d} ± 0.026

Each value [fibre composition and ash content for flour of control (C), water soaked (WS), alkali (0.1% NaOH) soaked (AS), hydrothermally treated for 5 min (H-5), hydrothermally treated for 10 min (H-10) and hydrothermally treated for 15 min (H-15) samples] is expressed as means ± standard deviation.

Table 4. Effect of pre-dehulling treatments on physical and functional properties of oat flour

Treatments	Bulk density (g/ml)	*L**	*a**	*b**	WAI (ml/g) [A]	WSI (%) [B]	OAC (ml/g) [C]
C	0.438^{bc} ± 0.010	81.79^{a} ± 0.10	-0.71^{b} ± 0.02	7.69^{a} ± 0.17	1.007^{a} ± 0.237	3.309^{a} ± 0.011	1.600^{b} ± 0.013
WS	0.379^{a} ± 0.013	83.56^{c} ± 0.36	-0.23^{d} ± 0.08	8.89^{bc} ± 0.05	1.588^{b} ± 0.019	4.499^{b} ± 0.045	1.517^{a} ± 0.025
AS	0.417^{b} ± 0.005	83.64^{c} ± 0.20	-1.91^{a} ± 0.00	8.47^{b} ± 0.27	1.255^{bc} ± 0.019	5.651^{c} ± 1.320	1.621^{b} ± 0.026
H-5	0.459^{c} ± 0.027	82.48^{b} ± 0.59	-0.53^{b} ± 0.14	9.09^{c} ± 0.55	2.431^{c} ± 0.104	4.647^{b} ± 0.165	1.628^{b} ± 0.020
H-10	0.503^{d} ± 0.009	82.92^{b} ± 0.36	-0.19^{d} ± 0.06	10.11^{d} ± 0.06	2.687^{d} ± 0.040	4.795^{b} ± 0.202	1.677^{c} ± 0.030
H-15	0.496^{d} ± 0.012	84.79^{d} ± 0.00	-0.48^{c} ± 0.01	9.65^{d} ± 0.085	2.940^{e} ± 0.074	5.026^{b} ± 0.533	1.736^{d} ± 0.022

Each value [fibre composition and ash content for flour of control (C), water soaked (WS), alkali (0.1% NaOH) soaked (AS), hydrothermally treated for 5 min (H-5), hydrothermally treated for 10 min (H-10) and hydrothermally treated for 15 min (H-15) samples] is expressed as means ± SD.
Values in the same column followed by different superscript letters are significantly different *($P \leq 0.05$)*
[A] Water absorption index; [B] Water solubility index; [C] Oil absorption capacity

High OAC (oil absorption capacity) is associated with the presence of non-polar side chains of flour protein which bind with hydrocarbon chains of oil (Adebowale et al., 2004). Higher OAC improves flavour and palatability of the products. WS samples had significantly ($P \leq 0.05$) lower OAC than control samples (1.6 g/ml). However, flour samples of hydrothermally treated grains had significantly higher OAC. Significant ($P \leq 0.05$) increase in OAC was observed with increase in steaming time during hydrothermal treatment of grains. Sharma et al. (2011) also obtained increased OAC during thermal treatment of barley grains.

3.5. Pasting properties

The pasting properties of oat flour, namely, PV (peak viscosity), FV (final viscosity), BDV (breakdown viscosity) and SBV (setback viscosity) are important to food processing and indicate cooking behaviour of starch in flour. PV, FV, BDV and SBV for control were 1885, 4327, 444 and 2886, cP, and corresponding values for all treated samples ranged 1076-1870, 2107-3827, 95-492 and 1044-2574, cP, respectively. Peak viscosity indicates maximum swelling capacity of starch, a major component of oat flour. Except for AS, all other treated samples had significantly ($P \leq 0.05$) low PV as compared to control (Figure 1). Partial depolymerization of starch by alkali may have caused lower PV and significantly ($P \leq 0.05$) low FV in AS flour samples as compared control. Significantly low pasting properties in WS samples may be due to activity of some enzymes during soaking. Further, there was significant decline in all pasting properties in hydrothermally treated samples as compared to control. Among the hydrothermally treated samples, significant ($P \leq 0.05$) decrease in PV was observed with increase in duration of steaming from 5 min (1472.667 cP) to 15 min (1076 cP). Soaking in water and subsequent steaming may have caused gelatinization of starch and leaching of amylose. Gujral et al. (Gujral et al., 2011) also reported lower PV and other pasting properties in pre-gelatinized oat starch. Ovando-Martínez et al. (Ovando-Martínez et al., 2013) attributed this behavior of starch to crystalline structure that remains intact after pre-treatment and makes it resistant to gelatinization and retrogradation. Further,

low tendency to retrograde makes it suitable for soups and beverages.

4. Conclusions

Pre-dehulling treatments such as soaking in water, alkali and hydrothermal treatments caused significant changes in composition of resulting flour. Results showed significant ($P \leq 0.05$) lowering of contents of indigestible components such as hemicellulose in flour of treated samples. Hydrothermal treatments of grain were effective in improving physical, functional and pasting properties of flour. In order to diversify use of oats in popular products such as bread, *chapatti* and noodles, there is need to prepare high quality flour. Treatments to locally available varieties of oat grains would expand scope for their use in varied food products and help to exploit the potential of multi-functional oat flour.

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OPTIMIZATION OF PROCESS PARAMETERS FOR EXTRACTION OF *Descurainia Sophia* SEED OIL WITH RESPONSE SURFACE METHODOLOGY

Yaqiang He [1], Giurgiulescu Liviu[2], Qian Lu [3]*

1. College of Food Science, Henan University of Technology, High-tech Industrial Development Zone, Zhengzhou, 450000, People's Republic of China

2. Technical University of ClujNapoca, North University Center of Baia Mare, Department of Chemistry and Biology, Victor Babes 62A, 430083, Romania

*3. Department of Food Science and Nutrition, College of Food, Agricultural and Natural Resource Sciences, University of Minnesota, Saint Paul, MN 55108, United States; *luxxx828@umn.edu*

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ABSTRACT

Descurainia Sophia with high oil content has been regarded as an alternative of traditional oil crops, such as peanut and soybean. In this study, surface response methodology was employed to optimize the process parameters for extraction of*Descurainia Sophia* seed oil. Four factors, type of extraction solvent, ratio of solid and liquid, extraction temperature, and extraction time, were investigated. The result indicated that petroleum ether is better for the extraction of *Descurainia Sophia* seed oil. The result of response surface showed that the optimum values forextraction time, temperature and ratio of solid and liquid were6 h, 60 ^{o}C, and 1:5, respectively. The high model *F* value (35.8) with a very low *P* value (< 0.0001) showed that this model is highly significant.

1. Introduction

The yield of oil-bearing crops is an important issue which can determine the production of edible oil. Under the impact of global climate change, in some areas, the yield of many oil-bearing crops decreased seriously (Schmidhuber & Tubiello, 2007). Furthermore, establishing agricultural infrastructure to improve the yield of oil-bearing crops would bring heavy financial burden to the government, particularly the government in undeveloped areas (Cosh and Cosh, 2011; Jiao Li and Li, 2013). So, developing the oil-bearing crops with low production cos is becoming more and more important.

Pistaciachinensis was investigated by researchers as a potential source of edible oil (Qin et al., 2012). However, the growth period of *Pistaciachinensis*is very long (Qin et al., 2012). This disadvantage seriously prevents the use of these crops in oil industry. Coconut oil was also regarded as a potential source of edible oil. However, the spread of coconut trees is limited to the Torrid Zone and the yield of coconut is not high (Lee et al., 2011). *Descurainia Sophia*, belonging to the *Brassicaceae* family, is widely spreaded in North America and Asia. The average yield of *Descurainia Sophia* growing on the barren land without agricultural irrigation system could reach 0.3 kg/m^2, which is equal to the yield of soybean (Li et al., 1997). Compared with the production of soybean and peanut, the production of *Descurainia Sophia* would not

utilize the agricultural infrastructure and fertile land (Luo, et al., 1997). So the production cost of *Descurainia Sophia*is very low while the average yield is similar to the yield of manytraditional oil-bearing crops. It was reported that the ratio of oil and dry biomass could reach 35% and the contents of linoleic acidand linolenic acid, which are positive to human health, in the seed oil of *Descurainia Sophia*were 15.9% and 31.7%, respectively (Li et al., 2010).Therefore, *Descurainia Sophia* can be regarded as a potential alternative to traditional oil-bearing crops. Not only the production cost, but also the processing cost is an important concern in the oil industry (Akinyemi et al., 2012). Improving the oil yield at low cost and low energy consumption is essential to the application of *Descurainia Sophia*in oil industry. Although the benefit of *Descurainia Sophia* was analyzed, to our knowledge, the research on the extraction process parameters was rare. The unreasonable extraction process would negatively impact the oil properties while the good extraction process is positive to the oil properties. Chemical leaching oil process now is replacing the traditional expelling process and becoming the major oil extraction technology (Abu-Arabi et al., 2000). In this study, three factors, ratio of solid and liquid, extraction temperature, and extraction time, in the chemical leaching oil process were investigated. Surface response methodology was employed to optimize the extraction process parameters.

2. Materials and methods

2.1. Materials

Descurainia Sophia was collected from the farm in Henan Province, P.R. China. Seeds were removed from the crop and broken into pieces. The sample was stored at 4^{o}C in dark until use. Petroleum ether, hexane, and hydrochloric acidwere obtained from Sinopharm Chemical Reagent Co, Ltd. (SCRC).

2.2. Content analysis

The contents of water, ash, crude protein and crude oil in *Descurainia Sophia* seeds were analyzed according to GB 5497-85, GB/T5505-1995, GB 5511-85 and GB 5512-85, respectively.

2.3. Extraction process

About20 g sample was mixed with certain volume of organic solventin a 100 mL flask. The volume of petroleum ether was adjusted to get certain ratio of solid and liquid. The extraction process was operated at certain temperature. When the extraction process finished, the solvent and oil were separated in the vacuum distillation system. The oil sample was stored at 4^{o}C in dark until analysis.

2.4. Selection of solvents for oil extraction

Petroleum ether, a by product of petroleum industry, has been used in the chemical leaching oil process for a long time because of its low price and good extraction capacity. Hexane is also an important extraction solvent used in food and chemical industry. So in this study the extraction capacities of petroleum ether and hexane for the *Descurainia Sophia*seed oil were evaluated. The extraction time, temperature and ratio of solid and liquid were set as 4 h, 40^{o}C and 1:3.The extraction capacities of two solvents were represented by oil extraction kinetics which was calculated using Equation 1:

$$Y_t = Y_0 e^{kt} \quad (1)$$

where: Yt is the percentage of extracted oil at time t; Y_0 is the percentage of total oil; k is the extraction constant and t is the extraction time (h). The extraction capacities of solvents were identified on the basis of extraction constant.

2.5. Optimization of extraction parameters

Three independent variables, the ratio of solid and liquid, extraction temperature, and extraction time, were studied respectively in the preliminary assay. The single-factor experimental design was shown in Table 1. Based on the result of preliminary assay, Box-

Behnken experiment was designed (Table 2 and Table 3). Response surface methodology was used to determine the impact of independent variables on the oil yield. A second degree polynomial equation (Equation 2) was employed to describe the extraction process mathematically, estimate the response of the dependent variable and predict the optimal point.

$$Y = a_0 + \sum_{i=1}^{n} a_i x + \sum_{i=1}^{n} a_{ij} x^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} a_{ij} x_i x_j \quad (2)$$

where: Y is the response variables; a_0 is a constant, a_i, a_{ii} and a_{ij} are the linear, quadratic and interactive coefficients, respectively; x_i and x_j are the levels of the independent variables. The goodness-of-fit of the regression model and the significance of parameter estimates were analyzed by Design Expert.

3. Results and discussions

3.1. Content analysis

The contents of water, ash, crude protein and crude oil in *Descurainia Sophia*seed were 5.07%, 4.22%, 25.47%, and 32.78%, respectively. The result indicated that the content of crude oil in *Descurainia Sophia* seed is as high as the crude oil content of some peanut species and soybean species. However, the production cost of *Descurainia Sophia* is much cheaper than that of peanut and soybean. This fact showed that *Descurainia Sophia* seed can be a potential alternative to traditional oil-bearing crops.

3.2. Solvent selection

When the extraction time, temperature and ratio of solid and liquid were 4 h, 50 ^oC and 1:3, the seed oil yields for petroleum ether and hexane were 23.65% and 21.81%, respectively. The extraction constants (k) of petroleum ether and hexane were -0.082 and -0.102, respectively. Therefore, for the extraction of *Descurainia Sophia* seed oil, petroleum ether is better than the hexane.

3.3. Preliminary assays

The extraction temperature and ratio of solid and liquid were set as 50^oC and 1:3, respectively. The impact of extraction time on the oil yield was shown in Figure 1a. The oil yield increased from 19.7% to 25.9% when the extraction time increased from 2 h to 6 h and then decreased to 25.1% when the extraction time increased to 8 h. Therefore, extracting the *Descurainia Sophia* seed for 6 h can get the highest oil yield. The ratio of solid and liquid and extraction time were set as 1:3 and 6 h, respectively. The extraction temperature was adjusted to analyze the oil yield (Figure 1b).The oil yield increased from 24.2% to 30.5% with the extraction temperature increasing from 40^oC to 60^oC. When the extraction temperature increased to 70^oC, the oil yield decreased to 29.1%. The reason for the decrease of oil yield is that high extraction temperature can cause the evaporation of petroleum ether and negatively impact the extraction capacity. Furthermore, high extraction temperature is unfavorable to the properties of oil, particularly the oil with high content of unsaturated fatty acids. This single-factor experiment indicated that to get highest oil yield the extraction temperature should be controlled at 60^oC. Ratio of solid and liquid (W/V) referred to the ratio of the *Descurainia Sophia* seed sample and volume of petroleum ether (g sample/mL petroleum ether). The extraction time and extraction temperature were set as 6 h and 60 ^oC. The result (Figure 1c) showed that the oil yields when the ratios of solid and liquid were 1:3, 1:4, 1:5 and 1:6 were 27.8%, 29.6%, 31.5% and 30.6%. Therefore, the oil yield reached the highest level, 31.5% when the ratio of solid and liquid was 1:5. The single-factor experiment indicated that the best extraction time, extraction temperature and ratio of solid and liquid should be 6 h, 60^oC, and 1:5. The Box-Behnken experiment was designed according to the result of single-factor experiment.

3.4. Result of Box-Behnken experiment

Factors and levels of response surface were shown in Table 2. The result of Box-Behnken experiment (Table 3), which covered 13 experiments, indicated that the oil yield reached highest level (31.7%) when the extraction temperature, extraction time and ratio of solid and liquid were 60^{o}C, 6 h and 1:5, respectively.

$$\text{Oil yield (\%)} = 31.7 - 0.775A + 2.05B + 1.25C + 0.15AB - 0.35AC - 0.2BC - 2.425A^2 - 1.975B^2 - 1.375C^2 \quad (3)$$

Table 1.Single-factor experimental design

Factor	1	2	3	4
Ratio of solid and liquid	1:3	1:4	1:5	1:6
Extraction temperature (^{o}C)	40	50	60	70
Extraction time (h)	2	4	6	8

Table 2. Factors and levels of surface response methodology

Factor	Level		
	-1	0	1
A: Extraction temperature (^{o}C)	50	60	70
B: Extraction time (h)	4	6	8
C: Ratio of solid and liquid	1:4	1:5	1:6

Table 3. Result of Box-Behnken experiment

No.	A	B	C	Oil yield (%)
1	0	0	0	31.7
2	0	-1	1	27.4
3	0	-1	-1	24.8
4	0	1	1	31.5
5	0	1	-1	29.7
6	1	-1	0	25.6
7	1	1	0	29.6
8	1	0	-1	28.1
9	1	0	1	30.2
10	-1	-1	0	25.3
11	-1	1	0	28.7
12	-1	0	-1	24.9
13	-1	0	1	28.4

Table 4. Variance analysis

Source	Sum of Squares	Degree of freedom	Mean Square	*F* Value	p-value Prob.> F
Model	106.1997	9	11.79997	35.83504	< 0.0001
A-Extraction temperature	4.805	1	4.805	14.59219	0.0065
B-Extraction time	33.62	1	33.62	102.0998	< 0.0001
C-Ratio of solid and liquid	12.5	1	12.5	37.96095	0.0005
AB	0.09	1	0.09	0.273319	0.6172
AC	0.49	1	0.49	1.488069	0.2620
BC	0.16	1	0.16	0.4859	0.5082
A^2	24.76053	1	24.76053	75.19466	< 0.0001
B^2	16.42368	1	16.42368	49.8767	0.0002
C^2	7.960526	1	7.960526	24.17513	0.0017
Residual	2.305	7	0.329286		
Lack of Fit	2.305	3	0.768333		
Pure Error	0	4	0		
Total variation	108.5047	16			

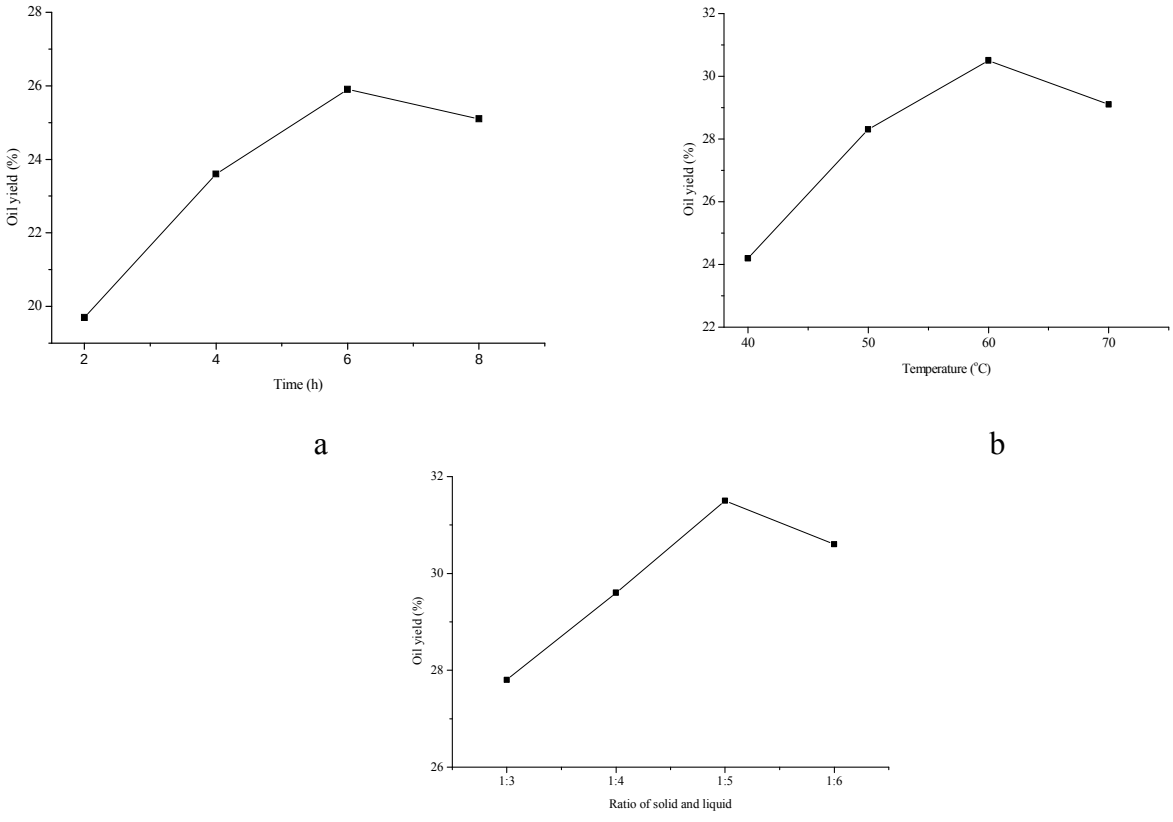

Figure 1. Impact of different conditions on the oil yield: (a) extraction time;(b) extraction temperature; (c) ratio of solid and liquid.

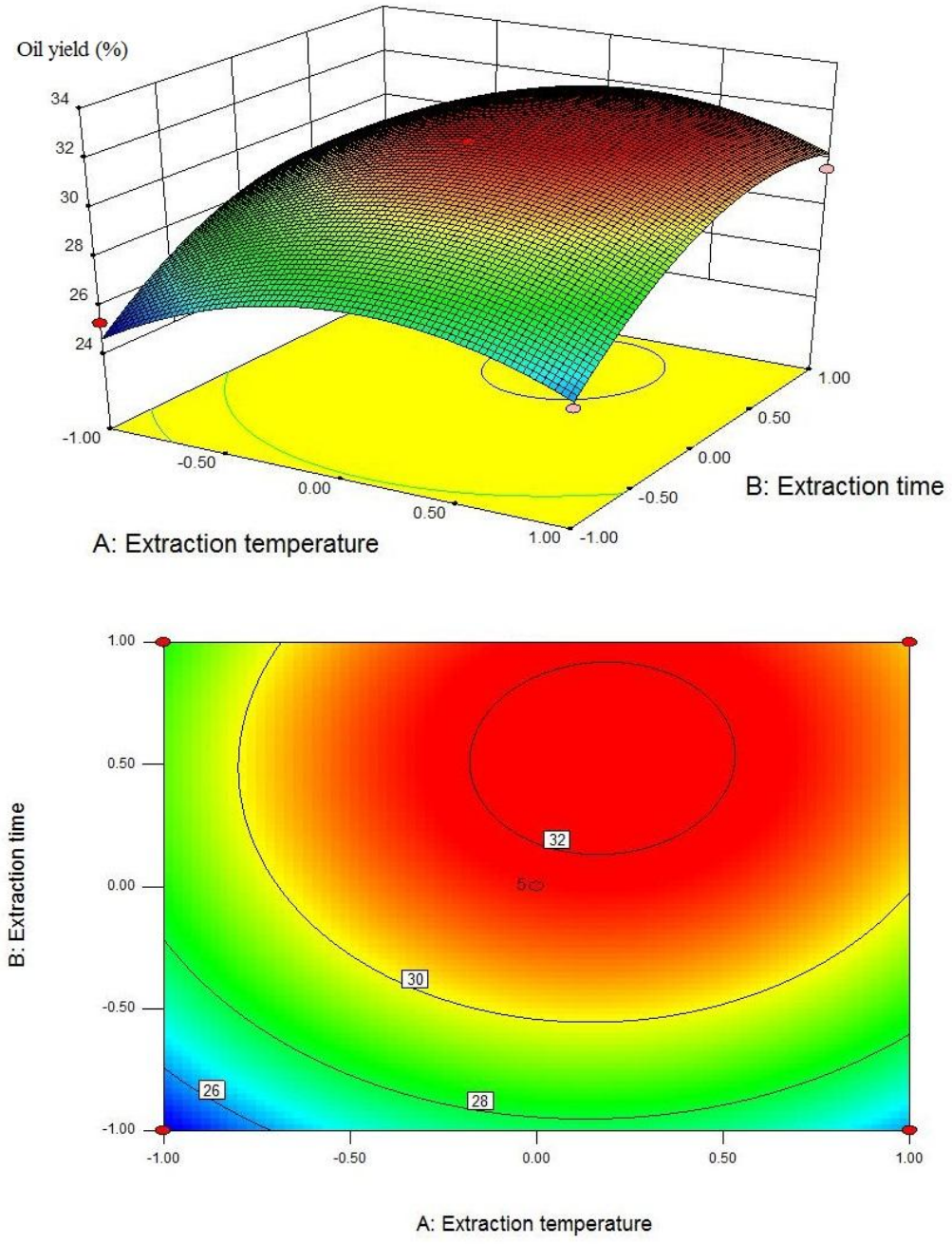

Figure 2. Response surface and contour plot for the effect of extraction temperature and time on the oil yield

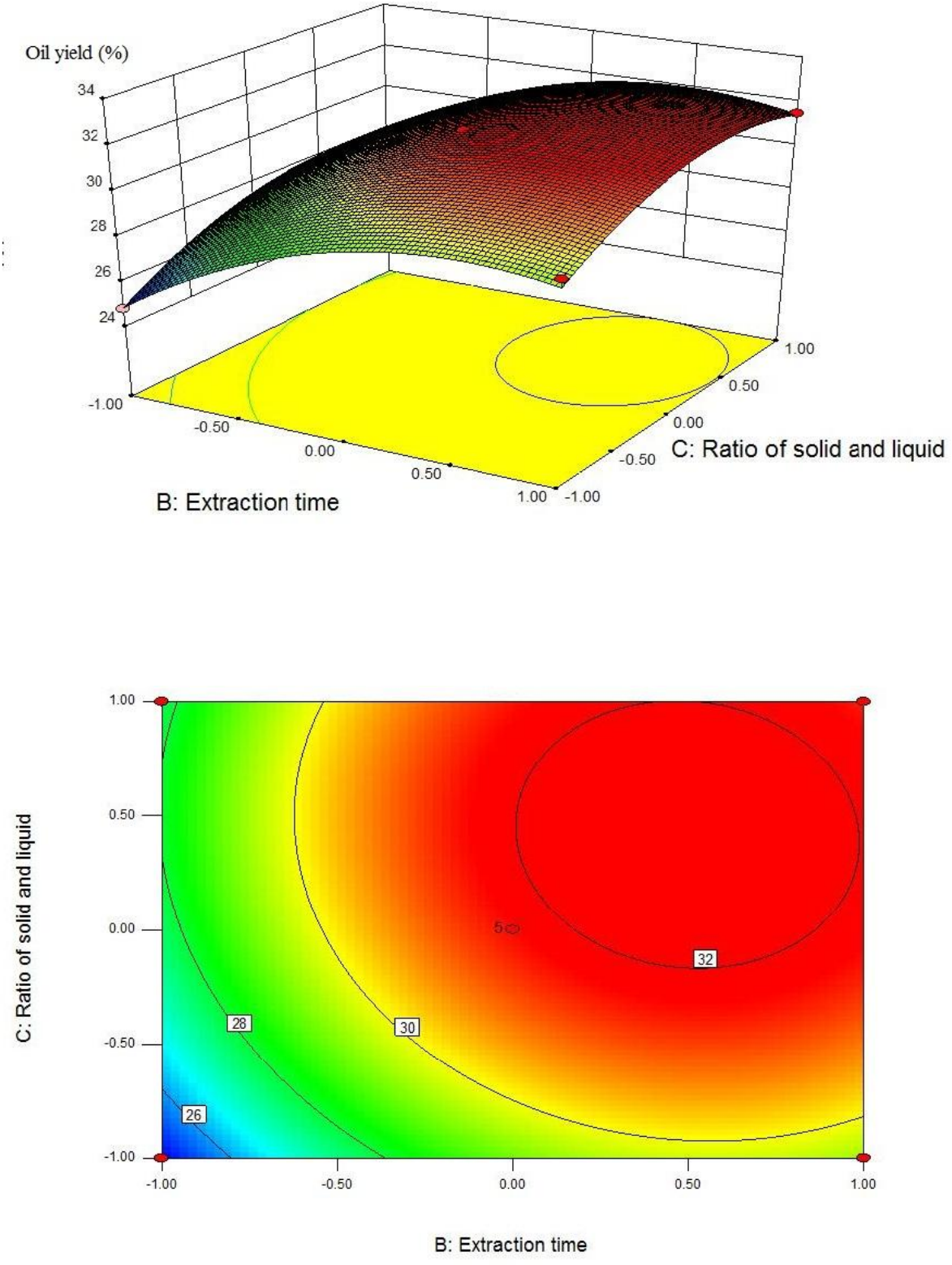

Figure 3. Response surface and contour plot for the effect of extraction time and ratio of solid and liquid on the oil yield

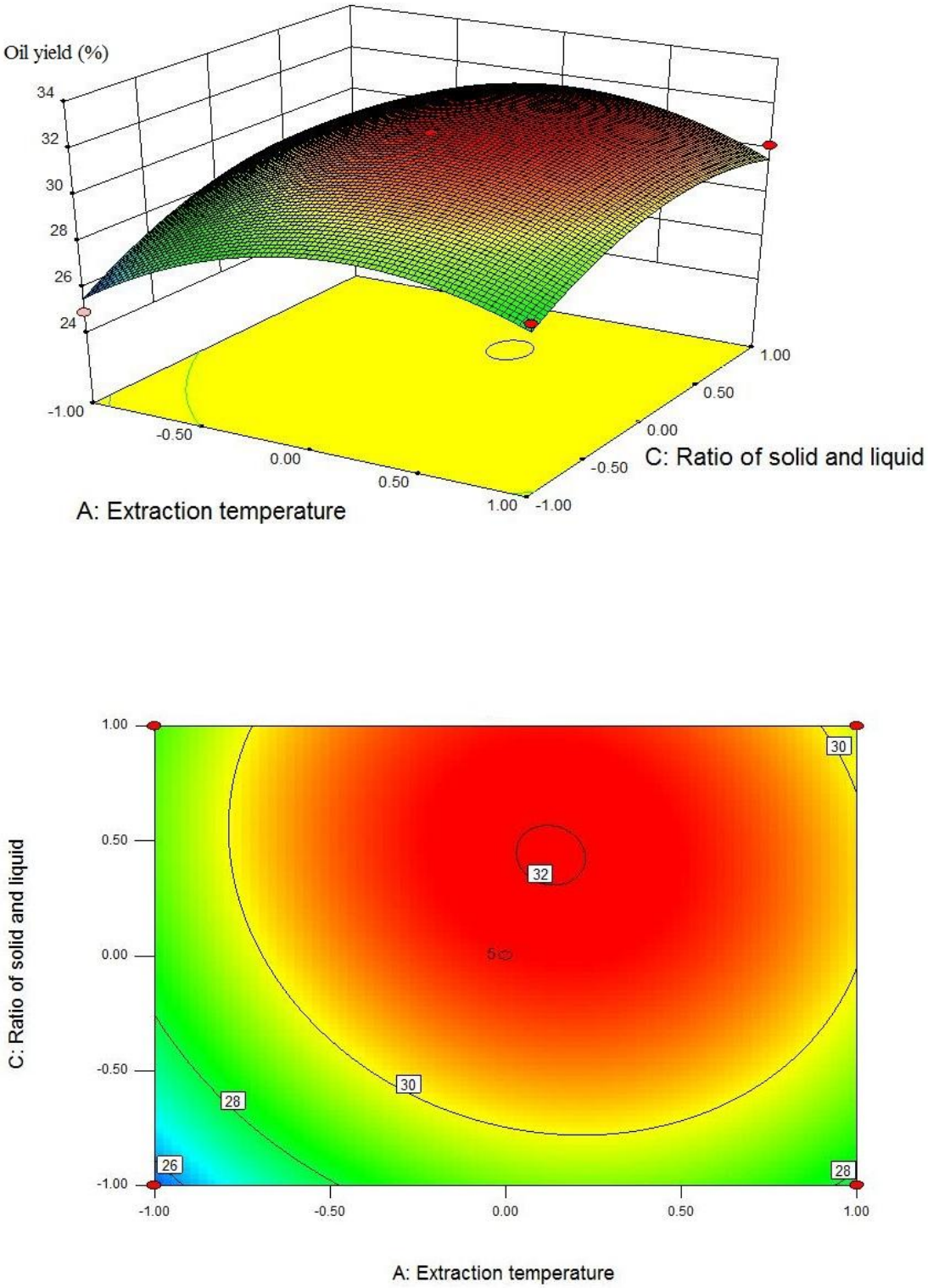

Figure 4. Response surface and contour plot for the effect of extraction temperature and ratio of solid and liquid on the oil yield

Regression analysis was performed to fit the response function. The second degree polynomial equation which reflects the relationship between oil yield and three independent variables is expressed as Eq.3, where the variables took the coded values. Equation 2 was used to predict the oil yield shown in Table 3. ANOVA analysis which is important to the determination of adequacy and significance of the model was shown in Table 4. The high model *F* value (35.8) with a very low *P* value (< 0.0001) shows that this model is highly significant. For terms A, B, C, A^2, B^2, and C^2, *P* values (< 0.01) indicates that these model terms are highly significant. However, some model terms, AB, BC, and AC, are not significant.

3.5. Response surface plot and optimization conditions

Equation 2 was used to generate the two-dimensional contour plot and three-dimensional response surface graph. It was reported that the three-dimensional response surface graph could determine the direction to take to increase a desired response while the two-dimensional contour plot could determine the levels of variables. Figure 2 is the response surface and contour plot indicating the effect of extraction temperature and time on the oil yield when ratio of solid and liquid is 1:5. An increase in the extraction temperature with corresponding increase in the extraction time can improve the oil yield. However, the result showed that high extraction temperature and long extraction time can reduce the oil yield. Furthermore, high extraction temperature can cause the deterioration of oil and negatively impact the oil property. There is no significant interaction between extraction temperature and extraction time in this model. Figure 3 is the response surface and contour plot showing the effect of extraction time and ratio of solid and liquid on the oil yield when the extraction temperature is 60^{o}C. Although an increase in the extraction temperature with corresponding increase in the ratio of solid and liquid can improve the oil yield, long extraction time can reduce the oil yield a little bit. In the industry, long extraction time would cost much more energy. So the extraction time should be controlled to reduce the cost and improve the oil yield. There is no significant interaction between extraction time and ratio of solid and liquid. Figure 4 is the response surface and contour plot showing the effect of extraction temperature and ratio of solid and liquid on the oil yield when the extraction time is 6h.With the increase of extraction temperature and ratio of solid and liquid, the oil yield increased. However, high extraction temperature can reduce the oil yield. Furthermore, high extraction temperature will improve the production cost of oil in the industry. The result shows that there is no significant interaction between extraction temperature and ratio of solid and liquid.

4. Conclusions

The results obtained from this study revealed that *Descurainia Sophia* with low production cost and high oil content is a potential source of vegetable oil. Petroleum ether can be used as an effective and cheap extraction solvent. Extraction time, temperature and ratio of solid and liquid are three important factors impacting the *Descurainia Sophia* extraction of seed oil. The optimum values for extraction time, temperature and ratio of solid and liquid were 6 h, 60^{o}C, and 1:5, respectively. Under the optimized extraction condition, the oil yield of *Descurainia Sophia* seed can reach 31.7%. The result of response surface analysis indicated that the model is significant while there is no significant interaction between extraction time, temperature and ratio of solid and liquid.

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CHEMICAL AND MICROBIOLOGICAL CHANGES DURING RIPENING OF IRANIAN SALT-SUBSTITUTED PROBIOTIC WHITE CHEESE

H. R. Gheisari*, M. Aminlari, N. Sabbagh and M. Moraveji

*Department of Food Hygiene, School of Veterinary Medicine, Shiraz University, P.O. Box 71345-1731, Shiraz, Iran; *ghaisari@shirazu.ac.ir*

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ABSTRACT

The objective of this study was to manufacture a probiotic cheese with partial submission of NaCl with KCl., Iranian white brine cheeses (4 trials) were made by varying processes such as partial submission of NaCl with KCl and use of probiotic adjunct culture on separate days. All types of cheeses were ripened at 13 °C for 2 weeks and at 6°C to the end of ripening period. Cheeses were analyzed for the compositional, microbiological, color and sensory characteristics as well as lipolysis and organic acid profile. Cheese of each trial was sampled at 1, 15, 30, 45 and 60 days during ripening. Results of this study showed that partial substitution of NaCl by KCl has no significant effect on the physicochemical and sensory properties, color, organic acids production and lipolysis of cheeses. Substitution provided positive effects on *L. delbrueckii subsp. bulgaricus* counts. Inclusion of probiotic microorganisms in the white brine cheese increased protein content and organic acids production and decreased lipolysis. In conclusion, Iranian probotic salt substituted cheese is a functional food with potential as to be used in patients with cardiac and renal diseases.

1. Introduction

Probiotics are living micro-organisms which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition (Guarner and Schaafsma, 1998). Foods containing such micro-organisms fall within the "functional foods" category and are described as foods claimed to have a positive effect on health (Anuradha and Rajeshwari, 2005). Functional foods should contain at least 10^7 CFU g^{-1} probiotic bacteria and should be consumed at levels higher than 100 g day^{-1} to have positive effects on health (Picard et al., 2005). Many different strains and species of lactobacilli and bifidobacteria have been used commercially as probiotics. It is well known that *Lactobacillus acidophilus* has health promoting effects and antagonistic activity against food-borne disease agents (Gilliand and Speck, 1977). *Lactobacillus acidophilus* is a probiotic microorganism available in conventional foods and dietary supplements. On the other hand, addition of adjunct cultures has shown great promise in manufacture of foods and improve their sensory quality (Mohebbi and Ghoddusi, 2008). Fermented dairy products are the best choice for probiotic microorganisms. Cheese may offer certain advantages over other products in terms of delivery of viable probiotics, such as the higher pH of the cheese, the higher fat content and more solid consistency of cheese which may offer protection to the probiotic in the gastrointestinal tract (Ong et al., 2006; Pouch Downes and Ito, 2001; Mazahreh et al., 2009).

The effect of salt and especially the uniformity of salt concentration, on the quality of cheese is well recognized. The roles of salt in cheese include control of microbial growth, modulation of the activity of enzymes, lowering the water activity of cheese and so on. In addition to its preservative effect, salt exerts a major influence on cheese composition, ripening rate, texture, flavor and quality (Guinee, 2004; Johnson et al., 2009). While the physiological requirement of Na^+ as a dietary constituent is universally accepted, there is growing concern that an excess (>~ 2.4 g/day for healthy adults) induces physiological defects, including hypertension, increased urinary calcium excretion, a higher risk of osteoporosis and occurrence of kidney stones (Kaplan, 2000; Heaney, 2006; Massey, 2005). Such concern has led to a recommendation for a reduced dietary intake of Na^+, classification of foods (high, medium, low sodium), declaration of sodium level on the food labels and an increased demand for reduced-sodium foods, including cheese (Morris and Dillon, 1992; Narhinen et al., 1998). Approaches to reduce the Na^+ level in cheese include: 1- reducing the level of added salt (Lindsay et al., 1985); 2- partial or complete substitution of NaC1 by other salts such as KC1, $MgCl_2$ or $CaC1_2$ (Katsiari et al., 1997; Salem and Abeid, 1997; Abou-E1-Nour, 1998); 3- a reduced salt level in combination with flavor enhancing substances such as autolyzed yeast extract (Demott et al., 1986); and 4- the use of ultrafiltration and reverse osmosis of retentate-supplemented milks to alter the mineral level in the cheese (Kosikowski, 1983, 1985; Kindstedt and Kosikowski, 1984; Lindsay et al., 1985). KCl has been the most widely and successfully used partial replacement for NaCl in cheese (Reddy and Marth, 1993).

Randomized trials have shown that increasing potassium intake in the diets is linked with lower cardiovascular mortality (He and MacGregor, 2008; He et al., 2010), lower blood pressure (He et al., 2005), and is likely to prevent or at least slow down the progression of renal diseases (He and MacGregor, 2008). In addition, potassium may promote renal calcium retention, resulting in a more positive calcium equilibrium (Lemann et al., 1993; Zhu et al., 2009). Overall, it seems that potassium contributes to greater bone mineral density, especially in elderly people (Whiting et al., 2002). Katsiari et al. (1997) reported that up to a 50% reduction of sodium content in Feta cheese is feasible, with partial replacement of NaCl by KCl, without an adverse effect on its quality.

White brined cheese is a major item in the Iranian diet and white cheese is a close-textured brined cheese, resembling Beyaz Peynir (Turkish white cheese) and feta but differs from feta in the way it is made. No research has been conducted on the manufacture of an Iranian dietary cheese with functional and probiotic effects. Thus, the objective of the present study was to manufacture a low sodium and probiotic Iranian cheese.

2. Material and methods

2.1. Treatments, cultures and rennet

Four types of cheeses were produced. Control cheese were made with regular starter cultures and brined with NaCl (A). Cheese made with regular starter cultures and probiotic adjunct culture and brined with NaCl (B). Two remained cheese treatments were like the ones described above but were brined with a 3:1mixture of NaCl/ KCl, w/w) (C, D). Two freeze- dried direct-to-vat cultures (YC-280 and La-5; Chr. Hansens Dairy cultures, Denmark) were used as starter. Culture YC- 280 contained *Streptococcus thermophilus* and *lactobacillus delbrueckii subsp. bulgaricus*. Culture La-5 contained *L. acidophilus*. As a coagulant, chymosin, derived by fermentation [Standard rennet, Chy-Max; Chr. Hansen Inc., Denmark; 2235 international milk clotting units (IMCU) mL^{-1} or 1-3gr/100 l milk) was used.

2.2. Cheese-making procedure

Iranian white brine cheeses were made on separate days. Pasteurized (72 °C×15 sec) cow milk was obtained from local dairy industries. For each trial, 10 kg milk was used. The milk

compositional and bacteriological characteristics are shown in Table 1. All types of cheeses were made triplicate according to the Iranian standard for white brine cheese. For cheese making, the milk was maintained at 35°C and pH was adjusted to 7.0. $CaCl_2$ was added at a level of 20 g 100 kg^{-1} of milk, followed by the addition of starter cultures 45 min before renneting. For conventional cheese, starter cultures` (*S. thermophilus* and *L. delbrueckii subsp. bulgaricus*) were used at a level of 0.01% (w/v) and for probiotic ones, milk was inoculated with starter cultures (*L. acidophilus* at a level of 0.02% (w/v)). Chymosin was added to each cheese vat at a level sufficient to coagulate the milk in 60 min (1-3 g 100 kg^{-1}). The curd was cut crossways into cubes of 3 cm and left for 10 min, followed by stirring and whey drainage. The curd was pressed in the vat using weights (10 kg) for 1 h and then cut to suitable size and packed in plastic containers. All cheeses were soaked in 20% (w/v) sterile brine (pH was adjusted to 7.0 ± 0.2) for 16 h and then replaced with 11% brine for 2 months. All types of cheeses were ripened at 13°C for 2 weeks and at 6 °C to the end of ripening period. Cheese of each trial was sampled at 1, 15, 30, 45 and 60 days during ripening.

2.3. Chemical analysis

Titrable acidity of milk was determined by the Dornic method. The fat, protein, solid non fat, lactose and freezing point were determined by a digital milk scan (Lactostar, FUNKE GERBER, 230V). pH of milk and cheese samples was measured using a digital pH-meter (CG 824, Germany). Cheeses were analyzed for moisture and dry matter content by vacuum-oven (AOAC, 2010). Salt and fat contents were determined by Volhard and Gerber methods, respectively. The ash content of cheese samples was determined by dry ash method and total protein content was determined by measuring total nitrogen using the Kjeldahl method and converting it to protein content by multiplying by 6.38. All chemical measurements were done in quadruplicate (AOAC, 2010).

2.4. Microbiological analysis of milk

Milk samples were analyzed for aerobic mesophilic bacteria and coliform bacteria using standard methods (Vanderzant and Splittoesser, 1992). Plate count agar (Merck, Germany) was used for enumeration of aerobic mesophilic bacteria. Plates were incubated aerobically at 30°C for 72 h. For the count of coliform bacteria, violet red bile agar (LAB M, Lancashire, BL96AU, UK) was used and incubated aerobically at 37 °C for 48 h.

2.5. Microbiological analysis of cheese

Ten grams of each cheese type was transferred to a sterile bag under aseptic conditions and homogenized in 90 mL sterile diluent containing 0.1% peptone and 0.9% NaCl in distilled water, pH adjusted to 7.0 ± 0.2 for 3 min using a lab blender 400 stomacher (Model No, BA6021, 230-250 volts, 50 Hz). Serial dilutions were prepared by adding 1-9 mL sterile peptone and NaCl (0.1% and 0.9%). Samples were tested for counts of starter cultures (*S. thermophilus* and *L. delbrueckii sub*sp. *bulgaricus*) and adjunct culture (*L. acidophilus*) using standard methods. *S. thermophilus* was enumerated on *Streptococcus thermophilus* agar (Merck, Darmstadt, Germany) and incubated at 37°C under aerobic condition for 48 h. *L. delbrueckii sub*sp. *Bulgaricus* was enumerated on pH-modified MRS agar (pH 5.2) Merck, Germany] and incubated at 45°C in an anaerobic jar with a Gas Generating Kit (Merck) for 72 h. *L. acidophilus* was enumerated on MRS-sorbitol (MRS-S) agar and incubated anaerobically at 37°C for 72 h. MRS-sorbitol agar used for the selective enumeration of *L. acidophilus* was prepared by adding 10 mL of membrane filtered sterile 10% solutions (w/v) of sorbitol (Merck) to 90 mL of molten MRS agar just before pouring.

2.6. Lipolysis

The level of lipolysis was assessed in samples of 1, 15, 30, 45 and 60 day-old cheeses by measuring the Acid Degree Value (ADV) and determination of total free fatty acids. The

ADV was determined by a modified procedure developed by Park and Lee (2006). Approximately 20 g of sample was grated, homogenized and mixed with 6 g Na_2SO4 and adequate amount of chloroform and filtered through Whatman study No.1. The ADV was determined on 25 mL of this extract that was titrated against the standard alcoholic 0.1N NaOH solution.

2.7. Production of organic acids

Production of lactic acid and acetic acid was determined using High Performance Liquid Chromatography in reversed phase mode (RP-HPLC) by a modified procedure developed by Akalin et al. (2002). Grated cheese samples (7.0 g) were added to 50 mL of mobile phase buffer (acetonitrile), homogenized, extracted for 1 h and centrifuged at 7000 g at 4°C for 5 min. The supernatant was filtered twice through a 0.2 μm membrane filter (Organe, Gryodisc CA-PC 1520012) and approximately 1 mL aliquot from each sample was stored at -20°C until analyzed. The HPLC system consisted of a liquid chromatography shimadzu LC-6A, equipped with a UV-VIS detector, Shimadzu SPD-6AV, a column oven model CTO-6A, a system controller model SCL-6A and computerized analyzing chroinatopack model C-R6A and a shimadzu reverse phase C18 column model (CLC-ODS) shim-pack. The system was used at room temperature (25 °C). UV-VIS detector was set at 214 nm. Organic acids were eluted from the column at flow rate of 1.2 mL min^{-1}. The chart speed was maintained at 1 cm min^{-1}. A mobile phase of HPLC grade $(NH_4)_2HPO_4$, 0.5% w/v and acetonitrile, 0.4% v/v (at pH 2.24 with H_3PO_4) was run through the column. HPLC grade reagents were used as standards (Merck Chemical Co). Solvents were degassed under vaccum. Both solvents and standard solutions were filtered through 0.2 and 0.45 μm membrane filters (Orange, CA-PC).

2.8. Sensory evaluation

An acceptance sensory panel evaluated randomly coded cheese samples. The panel consisted of 40 members, with an age range from 20-50 years. Panelists were the students of faculty of veterinary medicine and the employees of food hygiene and public health department of Shiraz University. All types of cheeses were evaluated for texture, flavor, odor, color and appearance by the consumer panel on a 5-point hedonic scale (1 = least liked to 5 = most liked). Cheese blocks were cut into standard, bite-sized pieces; each piece measured 1.5×1×1 cm. Cheese pieces were placed into airtight plastic containers and conditioned at room temperature for 2 h before evaluation. Crackers and water were offered without limit to panelists during testing for cleaning the palates (Foegeding et al., 2003).

2.9. Color analysis

The color of cheese samples at 1, 15, 30, 45 and 60 days of ripening periods was quantitatively determined using a Hunter lab colorimeter system (Hunter lab, DP-9000, Hunter Associates laboratory, Inc., Reston, VA), in which L, a and b values correspond to whiteness, redness and yellowness, respectively. Color measurements were performed in triplicate for each treatment at different sites.

2.10. Statistical analysis

Data were analyzed using Analysis Of Variance (ANOVA) of the General Linear Models procedure of the Statistical Analysis System software (SAS., 2005). Duncan's multiple range test was used to determine if significant differences existed among treatments. $p<0.05$ was considered as a level of significance.

3. Results

3.1. Compositional and physicochemical properties

The mean values for the compositional and physicochemical properties of various Iranian white brine cheeses are given in Table 2. The fat, protein and ash contents of manufactured cheeses were in the range of 16.45-19.05, 13.61-16.90 and 3.22-3.7%, respectively. The

moisture of all cheeses met the Iranian standard values of a maximum of 60% (ISIRI, 2002). There were no significant differences between cheeses for the levels of salt. As it is shown in Table 2, the pH contents of cheeses and their wheys were in the range of 5.50-5.98 and 5.81-6.26, respectively. The fat and protein content of manufactured cheeses decreased during ripening, while the ash, moisture, salt, pH of cheese and pH of whey increased. Of course, the It is to be noted that pH of whey decreased during the first 15 days of ripening and subsequently increased thereafter (Table 3).

3.2. Microbiological analysis

Bacterial count of Iranian white brine cheeses starter culture and probiotic adjunct culture (*L. acidophilus*) are shown in Table 4. *S. thermophilus*, *Lactobacillus delbrueckii* subsp *bulgaricus* and *L. acidophilus* counts were in the range of 8.03-8.71, 5.37-6.85 and 7.42-8.21 (log10 $cfug^{-1}$), respectively. The counts (log10 $cfug^{-1}$) of *S. thermophilus*, *Lactobacillus delbrueckii subsp. bulgaricus* and *L. acidophilus* (La-5) decreased during ripening (Table 5).

3.3. Production of organic acids

The metabolic activity of the microorganisms in cheeses was monitored by estimating the metabolic products, lactic and acetic acids (Table 6). The lactic and acetic acid contents of manufactured cheeses were in the range of 3.11-7.62 and 4.99-12.31 mg g^{-1}, respectively. The lactic and acetic acids contents of manufactured cheeses decreased during the first 30 days of ripening and increased there after towards the end of ripening period (Table 7).

3.4. Lipolysis

The extent of lipolysis in the manufactured cheeses, expressed Acid Degree Value (ADV), is shown in Table 6. The ADV of cheeses were in the range of 1.34-1.90. The Acid Degree Value (ADV) of cheeses increased during ripening period (Table 7).

3.5. Cheese opacity

Table 8 shows the results of color analysis of Iranian white brine cheese treatments. The L, a and b values of manufactured cheeses were in the range of 74.00 -78.05, -1.20-(-2.00) and 6.47-10.60, respectively. There were no significant differences in the a and b values of manufactured cheeses. The treatment D (probiotic culture with KCl) showed the lowest L value. Ripening time affected L, a and b values of cheese treatments, such that L and a values decreased during the ripening period, while b values increased (Table 9).

3.6. Sensory characteristics

The results of the sensory evaluation of cheese groups are given in Table 10. No significant differences were seen between odor, flavor and color scores. Probiotic culture had a negative effect on the texture of manufactured cheeses. It was decreased the appearance of KCl treatments.

4. Discussions

4.1.Compositional and physicochemical properties

As may be seen from Table 2, Partial substitution of NaCl by KCl has no significant effect on the compositional and physicochemical properties of cheeses. These results are in agreement with those reported by other investigators (Reddy and Marth, 1993; Aly, 1995; Rampilli et al., 1995; Ramadan, 1995; Ayyash and Shah, 2011a) in their studies on the effect of partial substitution of NaCl by KC1 on various cheese varieties (Cheddar, Prato, UF Feta-type, Caciotta, Domiati and Nabulsi). It can be attributed to the fact that, as stated by Kosikowski (Taylor, 1983) technically, K+ have the same effects as Na+ in the cheese making process.

No significant differences were observed among the cheeses with and without the addition of probiotic bacteria regarding salt, fat, ash and moisture content. The results showed that cheeses without probiotic have lower whey pH than cheeses with probiotic. Addition of probiotic increased the protein content in

cheese manufactured with NaCl and decreased pH in cheeses with partial substitution of NaCl by KCl. The composition of the cheeses was within the suggested ranges of Iranian white brine cheeses, with good quality and texture parameters (ISIRI, 2002). The results thus show that addition of probiotic microorganisms into white brine cheese has no significant effect on cheese composition (except for protein), which confirms the findings of Stanton *et al.* (1998) and Ayyash et al. (2012).

The fat and protein contents of manufactured cheeses decreased during ripening, while the ash, moisture, salt, pH of cheese and pH of whey increased. PH of whey decreased during the first 15 days of ripening and then increased towards the end of ripening period. When cheese placed in brine, there is a net movement of NaCl molecules (as Na^+ and Cl^-) from the brine into the cheese because of the osmotic pressure difference between the cheese moisture and the brine. Consequently, the water in the cheese containing dissolved materials such as acids and minerals diffuses out through the cheese matrix with a flux approximately twice that of NaCl so as to restore osmotic pressure equilibrium. The establishment of these dynamic phenomena increases the salt content of the cheese as they ripen. The ash content of cheese samples increased as the salt content increased, respectively. The fat content of cheese treatments decreased during ripening due to slight increase in moisture content and also lipolysis of fat. Hayaloglu et al. (2002) related the decrease in protein content of Turkish white cheese (Beyaz peynir) during ripening to diffusion of some proteolysis products from the curd into the brine. In agreement with the findings in this study, Ehsani et al. (1999) reported that the amount of total N and NPN in the brine increased during ripening of Iranian white cheese. The slow solubilization of colloidal calcium phosphate during ripening causes a slow increase in pH. The pH of whey increase due to the liberation of free amino acids and short peptides from the cheese curd (Lucey et al., 2003).

4.2. Microbiological analysis

In order to be considered as a functional food it should contain at least 10^7 $cfug^{-1}$ probiotic bacteria and should be consumed at levels higher than 100 g day^{-1} to have positive effects on health (Picard et al., 2005). All of the probiotic cheese treatments have the desired number of the micro-organisms. Substitution of NaCl with KCl had no significant effect on the bacterial count of *S. thermophilus* and *L. delbrueckii subsp. bulgaricus. L. acidophilus* and *L. delbrueckii subsp. bulgaricus* counts were higher in the cheese samples with NaCl and partial substitution of NaCl by KCl, respectively. As it is shown in substituted salt group, *L. acidophilus* had a synergistic a significant effect on the bacterial count of *L. delbrueckii subsp. bulgaricus*. This may be due to the lower pH of samples with probiotic adjunct culture than samples without this culture. The bacterial count (log_{10} $cfug^{-1}$) of *S. thermophilus*, *L. delbrueckii subsp. Bulgaricus* and *L. acidophilus* (La-5) decreased during ripening. Although *L. acidophilus* decreased until the end of the ripening period, it did not decrease below 10^7 and 10^6 cfu g^{-1}. As indicated earlier, it is necessary to maintain the viability of *L.acidophilus* at $>10^7$ cfu g^{-1} of cheese, to call the cheese probiotic (Picard et al., 2005).

All probiotic containing cheeses prepared in this study thus satisfied the criteria for a probiotic food product. *L. acidophilus* counts in all cheeses however decreased by one to two log cycle probably due to unfavorable conditions in the cheese, such as high salt, high pH (It is well known that lactobacilli grow best under acidic conditions, lack of fermentable carbohydrate and low ripening temperature (Ong et al., 2006; Kasımoglu et al., 2004). *L. bulgaricus* counts in all cheeses however decreased more (about one to three log cycle) than *S. thermophilus* (about two log cycle) due to increase in pH (Ong et al., 2006).

4.3. Production of organic acids

The main organic acids of all Feta and Feta like-type cheeses throughout ripening were

lactic and acetic acids. In this study, significant differences were observed between cheese samples with and without probiotic adjunct culture in lactic acid content which is higher in the samples with probiotic culture.

Table1. Chemical and bacteriological analysis of pasteurized milks used for cheese making [*]

Fat (%)	Protein (%)	Lactose (%)	SNF (%) [†]	FPP (%) [§]	pH (%)	Acidity (%)	Coliform count (log cfu mL^{-1})	Total count (log cfu mL^{-1})
3.03 ± 0.12	3.51 ± 0.05	5.21 ± 0.03	9.68 ± 0.04	-0.54 ± 0.00	6.81 ± 0.04	16.54 ± 0.74	0.19 ± 0.27	3.48 ± 0.29

[*] Each value in the table is the mean ± SD of four replications.
[†] SNF= solid non fat; [§] FPP= freezing point

Table2. Compositions of Iranian white cheeses in brine (%w/w) [*]

Cheese code [†]	Fat	Protein	Ash	Moisture	Salt	pH CH [§]	pH WH [§]
A	16.45 ± 2.63	13.61 ± 4.16 [a]	3.70 ± 0.63	52.63 ± 3.65	3.17 ± 0.67	5.81 ± 0.17 [b]	5.91 ± 0.35 [a]
B	19.05 ± 3.33	16.91 ± 2.65 [b]	3.22 ± 0.51	52.10 ± 1.72	2.94 ± 0.52	5.98 ± 0.28 [b]	6.27 ± 0.28 [b]
C	17.75 ± 3.16	14.32 ± 4.05 [a]	3.63 ± 0.58	52.41 ± 3.03	3.21 ± 0.69	5.85 ± 0.19 [b]	6.22 ± 0.24 [b]
D	17.30 ± 3.61	15.37 ± 3.04 [ab]	3.35 ± 0.84	53.19 ± 3.72	3.25 ± 0.74	5.51 ± 0.47 [a]	5.81 ± 0.42 [a]

[*] Each value in the table is the mean ± SD of four replications.
Means within the same column with different superscripts (a-d) differ significantly ($p<0.05$).
[†] A = Cheese with NaCl; B = Cheese with NaCl and probiotic culture; C = Cheese with NaCl/KCl; D = Cheese with NaCl/KCl and probiotic culture
[§] pH CH = pH of cheese, pH WH = pH of whey

Table 3. Effect of ripening time on composition of Iranian white cheeses in brine [*]

Ripening time (day) / Composition (w/w)	0	15	30	45	60
Fat	22.44 ± 1.63 [e]	18.63 ± 1.96 [d]	17.44 ± 1.55 [c]	15.75 ± 1.53 [b]	13.93 ± 1.22 [a]
Protein	20.13 ± 1.82 [c]	15.39 ± 3.07 [b]	14.73 ± 3.03 [b]	12.69 ± 1.83 [a]	12.43 ± 2.12 [a]
Ash	2.79 ± 0.62 [a]	3.13 ± 0.47 [a]	3.56 ± 0.54 [b]	3.93 ± 0.34 [b]	3.83 ± 0.55 [b]
Moisture	49.70 ± 2.57 [a]	51.50 ± 2.70 [ab]	52.80 ± 2.38 [bc]	53.60 ± 2.39 [cd]	55.03 ± 2.73 [d]
Salt	2.21 ± 0.37 [a]	2.90 ± 0.41 [b]	3.27 ± 0.37 [c]	3.58 ± 0.37 [d]	3.74 ± 0.35 [d]
pH CH [†]	5.53 ± 0.23 [a]	5.65 ± 0.32 [ab]	5.82 ± 0.33 [b]	5.86 ± 0.35 [b]	6.08 ± 0.21 [c]
pH WH [†]	6.05 ± 0.48 [ab]	5.80 ± 0.32 [a]	6.13 ± 0.15 [b]	6.09 ± 0.21 [b]	6.21 ± 0.51 [b]

[*] Each value in the table is the mean ± SD of four replications.
Means within the same row with different superscripts (a-e) differ significantly ($p<0.05$).
[†] pH CH = pH of cheese; pH WH = pH of whey

Table 4. Bacterial count (Log_{10} cfug^{-1}) of Iranian white cheese in brine starter cultures and probiotic adjunct culture*

Cheese code†	Bacteria		
	ST §	LB ¶	LA (La5) ‡
A	8.71 ± 0.77	5.58 ± 1.24 [a]	
B	8.60 ± 1.19	5.74 ± 1.82 [a]	8.21 ± 1.08 [b]
C	8.06 ± 1.03	5.37 ± 0.91 [a]	
D	8.04 ± 1.26	6.85 ± 1.12 [b]	7.42 ± 0.70 [a]

* Each value in the table is the mean ± SD of four replications.
Means within the same column with different superscripts (a-b) differ significantly ($p<0.05$).
† A = Cheese with NaCl; B = Cheese with NaCl and probiotic culture; C = Cheese with NaCl/KCl; D = Cheese with NaCl/KCl and probiotic culture
§ ST= *Streptococcus thermophilus*; ¶ LB= *Lactobacillus delbrueckii subsp. bulgaricus*;
‡ LA= *Lactobacillus acidophilus* (la-5).

Table 5. Effect of ripening time on bacterial count (log_{10} cfu g^{-1}) of Iranian white cheese in brine starter cultures and probiotic adjunct culture *

Ripening time (day) / Bacteria	0	15	30	45	60
ST §	9.46 ± 1.08 [c]	8.38 ± 0.54 [b]	8.11 ± 0.69 [b]	8.28 ± 0.69 [b]	7.12 ± 1.01 [a]
LB ¶	7.52 ± 0.76 [d]	6.24 ± 1.34 [c]	5.92 ± 0.86 [bc]	5.21 ± 1.06 [ab]	4.49 ± 0.83 [a]
LA (la5) ‡	8.31 ± 1.21 [b]	8.39 ± 0.57 [b]	7.87 ± 0.92 [ab]	7.77 ± 0.97 [ab]	6.93 ± 0.42 [a]

* Each value in the table is the mean ± SD of four replications.
Means within the same row with different superscripts (a-d) differ significantly ($p<0.05$).
§ ST= *Streptococcus thermophilus*; ¶ LB= *Lactobacillus delbrueckii subsp. bulgaricus*;
‡ LA= *Lactobacillus acidophilus* (la-5).

Table 6. Effect of treatment on organic acid concentrations (mg g^{-1}) and acid degree value of Iranian white cheeses in brine *

Cheese code † / Factor	A	B	C	D
Lactic acid	3.11 ± 1.79 [a]	7.63 ± 3.45 [b]	4.04 ± 3.84 [a]	7.60 ± 5.77 [b]
Acetic acid	5.33 ± 0.99 [a]	12.32 ± 2.92 [b]	5.50 ± 2.92 [a]	5.00 ± 0.62 [a]
Acid degree value	1.89 ± 0.68 [b]	1.44 ± 0.35 [a]	1.71 ± 0.40 [ab]	1.91 ± 0.56 [b]

* Each value in the table is the mean ± SD of four replications.
Means within the same row with different superscripts (a-c) differ significantly ($p<0.05$).
† A = Cheese with NaCl; B = Cheese with NaCl and probiotic culture; C = Cheese with NaCl/KCl; D = Cheese with NaCl/KCl and probiotic culture

Table 7. Effect of ripening time on organic acid concentrations (mg g^{-1}) and acid degree value of Iranian white cheeses in birne [*]

Ripening time (day) / Factor	0	15	30	45	60
Lactic acid	11.12 ± 6.07 [b]	3.83 ± 1.99 [a]	3.01 ± 1.49 [a]	4.50 ± 2.62 [a]	5.51 ± 2.82 [a]
Acetic acid	9.53 ± 2.94 [b]	6.38 ± 2.72 [ab]	5.61 ± 2.56 [a]	8.21 ± 2.61 [ab]	8.48 ± 6.71 [ab]
Acid degree value	1.20 ± 0.18 [a]	1.63 ± 0.49 [b]	1.67 ± 0.41 [bc]	1.96 ± 0.44 [cd]	2.22 ± 0.53 [d]

[*] Each value in the table is the mean ± SD of four replications.
Means within the same row with different superscripts (a-d) differ significantly ($p<0.05$).

Table 8. Color parameters of Iranian white cheeses in brine [†]

Scales / Cheese code[§]	L*	a*	b*
A	77.68 ± 4.20 [b]	-1.26 ± 1.33	8.84 ± 5.97
B	78.05 ± 3.73 [b]	-1.20 ± 1.19	10.60 ± 6.85
C	77.35 ± 3.96 [b]	-1.75 ± 1.02	10.45 ± 5.81
D	74.00 ± 5.02 [a]	-2.00 ± 1.41	6.47 ± 4.35

[†] Each value in the table is the mean ± SD of four replications.
Means within the same column with different superscripts (a-b) differ significantly ($p<0.05$).
[§] A = Cheese with NaCl; B = Cheese with NaCl and probiotic culture; C = Cheese with NaCl/KCl; D = Cheese with NaCl/KCl and probiotic culture

Table 9. Effect of ripening time on L, a and b color parameters of Iranian white cheeses in brine†

Ripening time (day) / Scales	0	15	30	45	60
L*	81.40 ± 2.53 [c]	79.38 ± 3.22 [c]	76.43 ± 2.47 [b]	73.13 ± 3.59 [a]	74.06 ± 3.86 [a]
a*	-1.27 ± 0.80 [b]	0.06 ± 1.24 [c]	-2.14 ± 0.36 [a]	-2.06 ± 1.06 [a]	-2.38 ± 0.72 [a]
b*	0.87 ± 0.83 [a]	4.63 ± 1.20 [b]	13.14 ± 3.70 [c]	12.47 ± 2.39 [c]	15.06 ± 2.32 [d]

[†] Each value in the table is the mean ± SD of four replications.
Means within the same row with different superscripts (a-d) differ significantly ($p<0.05$

Table 10. Sensory evaluation of different Iranian white cheese groups at the end of ripening period (60 day) [*]

Cheese code [†] / Factor	A	B	C	D
Texture	4.40 ± 0.67 [b]	3.57 ± 0.87 [a]	4.27 ± 0.75 [b]	3.78 ± 0.75 [a]
Flavor	3.50 ± 0.88	3.18 ± 0.75	3.13 ± 0.79	3.30 ± 0.94
Odor	3.50 ± 0.88	3.50 ± 0.72	3.65 ± 0.83	3.70 ± 0.85
Color	4.25 ± 0.74	3.98 ± 0.70	4.25 ± 0.63	4.16 ± 0.76
Appearance	4.20 ± 0.72 [ab]	3.85 ± 0.80 [a]	4.28 ± 0.72 [b]	3.86 ± 0.82 [a]

[*] Each value in the table is the mean ± SD.
Means within the same row with different superscripts (a-d) differ significantly ($p<0.05$).
[†] A = Cheese with NaCl; B = Cheese with NaCl and probiotic culture; C = Cheese with NaCl/KCl; D = Cheese with NaCl/KCl and probiotic culture

This may be due to the presence of *L. acidophilus* that produce more lactic acid in the treatments with probiotic culture. These results are consistent with other studies (Manolaki et al., 2006; Katsiari et al., 2002).

Acetic acid is considered as a product of several biochemical pathways, such as fermentation of lactate and citrate or metabolism of amino acids by bacteria. It contributes greatly to the final flavor of Feta cheese (Abd El-Salam and Alichanidis, 2004; Kandarakis et al., 2001; Kondyli et al., 2002). Acetic acid content of cheese samples with NaCl and probiotic culture was significantly higher than the samples without culture. This is due to the higher amount of free amino acids in cheeses with adjunct culture (Michaelidou et al., 2003), which might have served as precursors for the formation of acetic acid.
Substitution of NaCl with KCl had no significant effect on lactic and acetic acids contents of cheese samples. In the study of Ayyash and Shah (2011b), the effect of substitution of NaCl with KCl on chemical composition of low-moisture Mozzarella cheese, organic acids profile did not differ between salt treatments at the same storage time. The lactic and acetic acids contents of cheeses decreased during the first 30 days of ripening and then increased to the end of ripening period. These results are in agreement with other studies (Ong et al., 2006). This variation could be due to the amount of free amino acids in cheeses which might have served as precursors or prevalence of other metabolic pathways leading to consumption of these acids.

4.4. Lipolysis

Substitution of NaCl with KCl had no significant effect on the rate and extent of lipolysis in the cheese samples. Addition of probiotic decreased acid degree value in cheeses manufactured with NaCl. Katsiari and Voutsinas (1994) and Katsiari el al. (2000) reported no significant differences ($P>0.05$) in the levels of total FFA of Feta cheeses made with NaC1, KCl, or various mixtures of these two salts. The Acid Degree Value (ADV) of cheeses increased during ripening period. The increase in the ADV could be due to the lipolysis that occur to some extent and formation of simple nitrogen compounds, especially free amino acids, which might serve as precursors for the formation of volatile fatty acids (Aly, 1994). The ADV may be a good index of cheese ripening (Katsiari et al., 2001).

4.5. Cheese opacity

Substitution of NaCl with KCl and probiotic usage did not have significant affect on the a* and b* values in the samples. Cheese

samples manufactured with probiotic and partial submission of NaCl with KCl showed the lowest L* values. Kaya (2002) showed that whiteness of the Gaziantep cheese samples affected with changing the salt concentration in brine while L-value decreased with increasing salt concentration. Ripening time affects L*, a* and b* values of cheese treatments, in the manner that L* and a* values decreased significantly during the ripening period, but b* values increased. In the study of Marchesini et al. (2009), Asiago cheese ripening significantly influenced colour, resulting in a linear decrease of L* through the time of aging. In the case of b* it was observed a major decrease during the first year and after 18 month. A significant reduction in redness (a*) occured but only in the first year. According to the literature (Dufosse et al., 2005; Pinho et al., 2005), cheese ripening can lead to decrease in L*, and increase in b*; the difference in the results was probably due to the ripening time and to the degree of lipolysis which appeared to be much higher, as seen in other hard cheeses. L* showed a negative correlation with the protein percentage, while there was no correlation with the fat content and a* and b* were both negatively related to fat content.Khosrowshahi et al. (2006) reported that the progressed ripening significantly ($p<0.05$) decreased the whiteness of Iranian white cheese.

4.6. Sensory characteristics

Substitution of NaCl with KCl had no significant effect on the sensory scores. These results are in agreement with Katsiari et al. (1997) who reported that the mean scores for appearance, body and texture, flavor, and overall quality (total score) of cheeses salted with the NaCl/KCl mixtures were not significantly different from those of the control cheese at all sampling ages.

Also the addition of adjunct culture significantly decrease the texture scores of manufactured cheeses and appearance scores of cheeses with partial substitution of NaCl with KCl. The differences in grading scores may be attributable to differences in proteolysis, the levels of which showed the same trend as the grading scores. Ayyash et al. (2012) reported no significant differences in sensory attributes, including creaminess, bitterness, saltiness, sour-acid, and vinegar taste among Akawi cheeses with probiotic and partial substitution of NaCl with KCl at the same storage period.

5. Conclusions

In conclusion, the results of this study showed that the Iranian probotic substituted salt cheese can be considered a functional food. Partial substitution of NaCl with KCl and addition of adjunct culture did not significantly affect the physicochemical, microbiological and sensory characteristics of Iranian cheese. Therefore these products can be included in diets as a healthy food especially in cardiovascular and renal diseases.

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OPTIMIZATION OF PECTIN ISOLATION METHOD FROM PINEAPPLE (*ANANAS COMOSUS* L.) WASTE

Rajibul Karim[1], M. Burhan Uddin[1], Md. Fahad Jubayer[1]*

[1]*Department of Food Technology and Rural Industries, Bangladesh Agricultural University, Mymensingh- 2202, Bangladesh; *fahadbau21@hotmail.com*

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ABSTRACT
Acid extraction followed by ethanol precipitation was used to extract pectin from pineapple (*Ananas comosus* L.) peel in the present work. The yields of pectin at different temperature, pH, and time were determined. The pineapple peel was treated separately with different pH (1.5, 2.0, and 2.5), temperature (70, 80, and 90^{o} C) and time (20, 40, 60, 80, and 100 minutes). The result showed that yield varies from 3.88 to 13.06% in dry basis. Two factors that affect the extraction yield of pectin (temperature and pH) were systematically investigated by response surface analysis. Further, the two factors were optimized and their significance was analyzed. As a result, the optimal conditions for pectin extraction were determined as temperature 82.63^{o} C, pH 1.83, and time 65 minutes. Under these conditions, the actual pectin yield was 13.781%, which was close to the predicted value of 13.812% and the optimized process may provide useful guidance for industrial pectin preparation.

1. Introduction

The pineapple (*Ananas comosus* L.) is a one of the popular and delicious fruit in Bangladesh. According to cultivated area and yield of production it occupies the 4th position (Hasan et al., 2010). The main varieties that grew better in Bangladesh are Honey Queen, Giant Kew, and Ghorashal.

The world production of pineapple shows a steady increase over the years much of the expansion of pineapple industry in the developing countries of Far East, Africa and Latin America (Burkill, 1997). But in Bangladesh the production is poor. Its cultivation is confined within a limited area such as Madhupur, Chittagong Hill Tracts, and Sylhet only. The statistical data shows that average yields of pineapple are 48.06t/ha and 33.67 t/ha per year for Madhupur and Sylhet respectively (BARI, 2010).

With the increase in production of processed fruit products, the amount of fruit wastes generated is increasing enormously. Large amount of these wastes poses the problem of disposal without causing environmental pollution. These wastes can be effectively disposed by manufacturing useful byproducts from them. A valuable byproduct that can be obtained from pineapple peel is pectin. A research reported that postharvest losses in pineapple are 40 percent due to poor handling and indiscriminate use of growth promoting and ripening agents (Hassan, 2010).

Pectin occurs as a white to light brown powder or granular, and odorless or has slightly characteristic odor. Natural polymer like pectin is easy to isolate and purify, it is non-toxic and bio-compatible. Fruit peels of Citrus such as orange, lemon and lime, are well recognized as conventional sources of commercial pectin

(Rolin et al., 1993). Classically, two main production steps of pectin include extraction from raw material with water and isolation of pectin from the extracted solution by precipitation with alcohol (May, 1990; Voragen et al., 2003; Kalapathy and Proctor, 2001; Joye et al., 2000). Commercial pectin is extracted at high temperature by hydrolyzing proto pectin using acid (May, 1990; Minkov, 1996). In general, higher yield is obtained from high temperature and low pH extraction. In contrast, molecular weight and degree of esterification (DE) will be decreased (Joye, 2000). Therefore, the suitable condition for each kind of raw material needs to be optimized.

Pectin is widely used in food and pharmaceutical industries. The main use for pectin is as a thickening agent, gelling agent and stabilizer in food (May, 1990). Pectin also reduces syneresis in jams, jellies and marmalades and increases the gel strength of low calorie jams. Pectin is used in confectionery jellies to give a good gel structure, a clean bite it confers a good flavor release. Pectin can also be used to stabilize acidic protein drinks, such as drinking yogurt, to improve the mouth-feel and the pulp stability in juice based drinks and as a fat substitute in baked goods. Typical levels of pectin used as food additives are between 0.5 and 1.0% - this is about the same amount of pectin as in fresh fruit (May, 1990). Its medical uses are anti-diarrhea, detoxification and blood glucose lowering (Voragen et al., 2003).

Under the above circumstances the present investigation was planned to select a suitable method for pectin extraction from pineapple wastes, to optimize the temperature and pH for extraction of pectin as well as to prepare pectin powder from pineapple wastes.

2. Materials and Methods

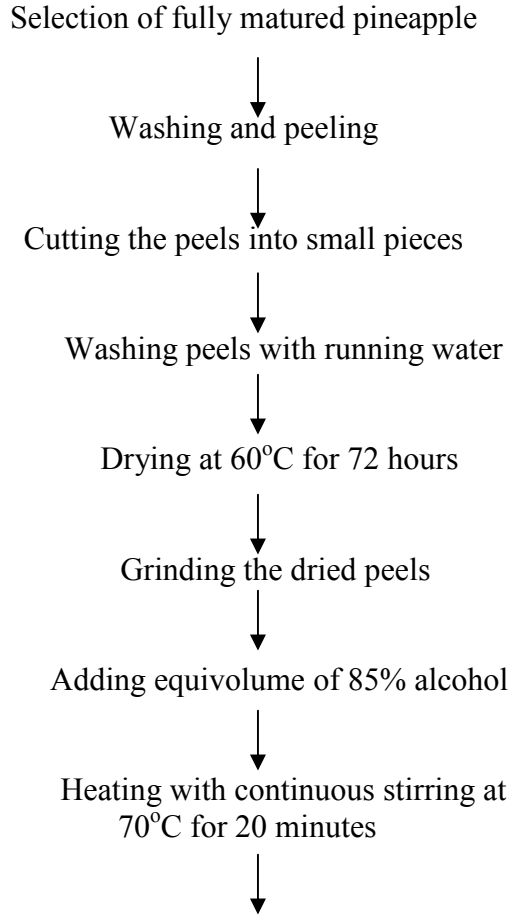
The investigation was conducted in laboratories of Department of Food Technology and Rural Industries, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Moisture content of raw sample was determined adopting AOAC (AOAC, 2005) method.

Sample Preparation

Fresh pineapples were washed with clean water and peeled with knife. The peels were cut into small pieces for easy washing and drying. After that it was washed with running water until sugar and other foreign materials removed. Then it was placed into a dryer at 60^{o}C for 72 hours. Then it was grinded to prepare fine powder. To prepare alcohol insoluble residue (AIR) sample it was treated with equivolume of 85% alcohol at 70^{o}C for 20 minutes. Then it was dried again at 55^{o}C until constant weight achieved. After that it was grinded again and sealed in a poly bag. Then it was stored into desiccators (Khule et al., 2012).

Flow sheet for alcohol insoluble residue (AIR) sample preparation



Drying at 55°C until constant

↓

Weight achieved

↓

Grinding and sealing

↓

Stored into a desiccators

Extraction Procedure

The extraction process was done as per the method described by Khule et al. (2012).

Procedure

At first 5g sample was taken into a 250 mL beaker and 150 mL distilled water was added. Then pH was adjusted at 1.5, 2.0, and 2.5. Then it was heated at 70, 80, and 90°C separately for 60 minutes. After heating the mixture at specified pH and temperature the sample was removed from the mixture by filtration through cloth. For easy coagulation filtrate volume was reduced to 25% by gentle heating and cooled at 4°C. After that filtrate was treated with an equal volume of 95% pure ethanol and left for 1 hr. After addition of ethanol brown colored pectic substance was precipitated. Then it was separated by mechanical filtration using Hoffman no.1 filter paper. Again pectin was washed with 70% acidic ethanol, 70% and 96% ethanol to a neutral pH. Then pectin was poured into a petridish and heated at 40°C overnight. Finally dried pectin was weighted and stored in airtight packet.

Yield of pectin (%) was calculated using following Equation 1:

$$\%\text{Yield of pectine} = \frac{\text{Weight of dried pectine}}{\text{Weight of sample}} 100 \quad (1)$$

Flow sheet for extraction of pectin

Sample 5g

↓

Adding 150mL distilled water

↓

Adjusting pH (1.5, 2.0 and 2.5) with 0.5N

↓

H_2SO_4

↓

Heating at (70, 80 and 90°C) with continuous stirring for 1hr

↓

Filtering the extract through cloth

↓

Reducing the filtrate volume to 25% by gentle heating & cool at 4°C

↓

Coagulating the filtrate using an equal volume of 95% ethanol & left 1hr

↓

Separating by filtration

↓

Washing with 70% acidic ethanol (5% HCl)

↓

Washing with 70% ethanol to a neutral pH

↓

Finally washing with 96% ethanol

↓

Drying at 40°C overnight

3. 3.Results and discussions

3.1. Yield of Pectin

The moisture content of raw pineapple peel was 85% (wb). The yield of pectin of pineapple peel differed from 5.16 to 13.06% in dry basis depending on different extraction conditions. Extraction of pectin was highly dependent on pH, temperature, and time. This experiment showed that pectic substances degrade in high pH, temperature (above 84°C) and time (above

60 minutes). Again extraction of pectin was reduced in low temperature and time. So optimization was required for maximizing the yield of pectin at optimum pH, temperature, and time. Response surface method was used for optimization.

3.2. Effect of pH

One of the most important parameters in pectin extraction procedure was pH (Pagan and Ibarz, 1999; Pagan et al., 2001). According to Figure 1 pH was not constant during experiment stages. The regulation of initial pH was achieved by adding suitable amount of 0.5N H_2SO_4. The figure shows that different variations occur during pectin extraction. Initially the yield of pectin increased with the increase of pH. It continued up to pH 1.83. Again yield of pectin felled with the increase of pH. Moreover pH had different effects on the extraction of pectic substances. According to some (Voragen, 2003) studies, pectin molecules can be partially soluble from plant tissues without degradation using weakly acidic aqueous solvents, but some pectin fractions are not extractable in this way. This is due to the attachment of them to other cell wall components. To extract these forms of pectin (proto pectin), acid hydrolysis was performed. On the contrary, it had reduced the molecular weight (pectin molecule molar mass) and hence the pectin molecules' proneness to precipitation. Both of these effects contrarily influence the yield, especially during long extraction.

3.3. Effect of Temperature

Temperature has a great influence on pectin extraction. In this experiment pectin was extracted at 70, 80 and 90^{o}C temperature. In the Figure 2, the curve shows that the pectin yield increases at first then goes down with the increase of temperature. At about 83^{o}C, pectin yield reaches the peak, because the temperature interval 80 to 85^{o}C is propitious to pectin hydrolysis. Higher temperature may lead to the cracking of pectin and the yield will decrease accordingly. When the temperature exceeds 85^{o}C, pectin yield goes down rapidly for the reason that under this temperature pectin is easy to be cracked. Thus, the temperature can select from 80 to 85^{o}C.

3.4. Effect of Time

It is essential to set an optimum extraction time. In this investigation different time (20, 40, 60, 80 and 100 minutes) were set at pH 2.0 and temperature 80^{o}C. Figure 3 exhibits that the product of pectin increases at the beginning and goes down after the peak point. When the extraction time is about 65 minutes, the pectin yield is high. In the process of pectin producing, the time between adding acid and being precipitated by ethanol should be as shorter as possible. The acid can destroy glycoside bond and ester bond. The destructive augment with the increase of extraction time. The consequence is that the molecule weight of pectin descends and the gelling property declines. Considering the consumption of energy, long time extraction goes against saving energy. In summary, the extraction time should be from 60 to 70 minutes, in which pectin can be extracted completely and the gelling property can be kept good.

3.5. Optimization of pectin extraction

RSM (Response Surface Method) was used to determine the optimum condition for pectin extraction from dried pineapple peels on the basis of dependent variable experiment. The dependent variables were extraction temperature and pH. The interaction effects of central factors (pH and temperature) are shown in Figure 4 as well as contours of estimated response surface is shown in Figure 5. Figure shows that the yield of pectin increases and then goes down with the increase of pH whose effect is significant. It also indicates that yield goes up with the increase of temperature. The reason is that at low temperature, the main reaction of pectin is decreasing and with the temperature rising, the hydrolysis reaction of glycosidic bond speeds up. However, if the temperature is too much high, it is easy for pectin to be demethylated and cracked. Pectin yield decreases with the increase of pH when extraction temperature is from 70 to 90^{0}C.

Yield of Pectin (%) = -342.684 + 7.3266 × Temperature + 58.7293 × pH - 0.0419667 × Temperature^2 - 0.2134 × Temperature × pH - 11.2187 × pH^2

(2)

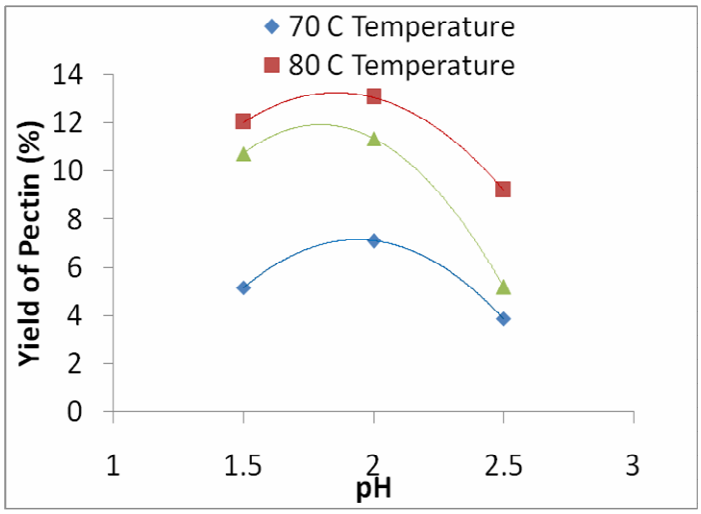


Figure 1. Effect of pH on pectin extraction at different temperatures

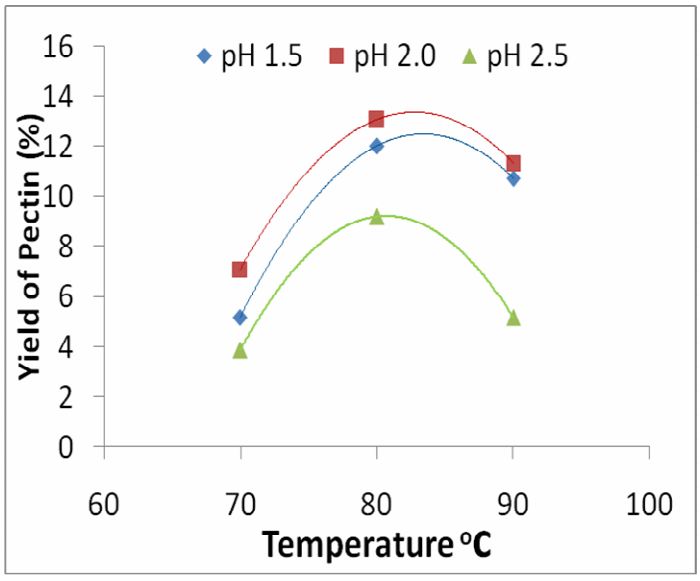


Figure 2. Effect of temperature on pectin extraction at different pH

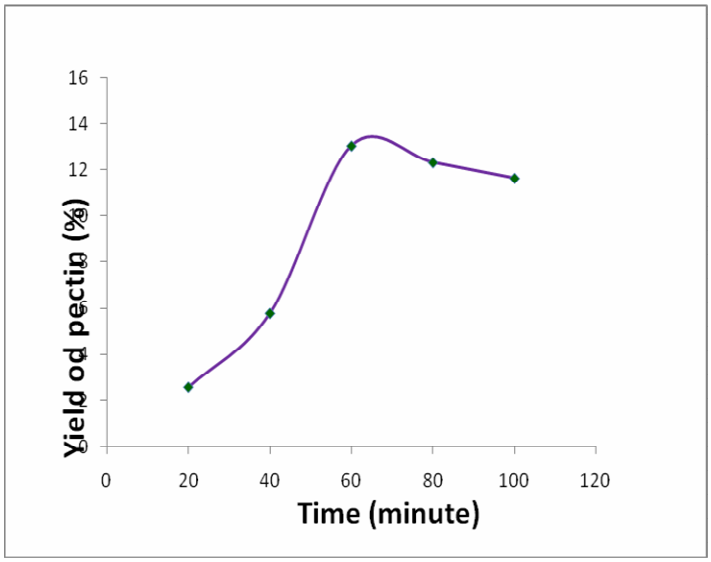


Figure 3. Effect of time on pectin extraction at pH 2.0 & temperature 90^{o}C

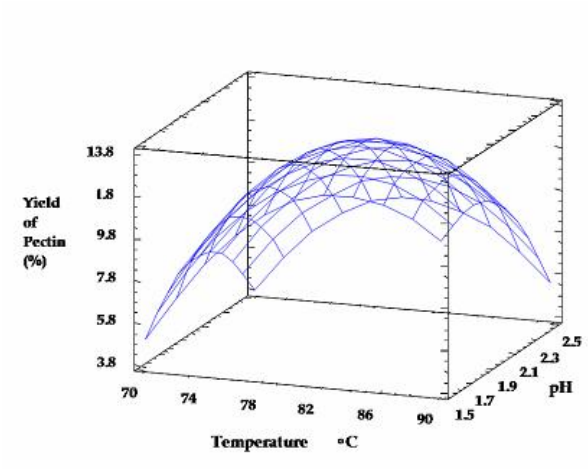


Figure 4. Estimated response surface

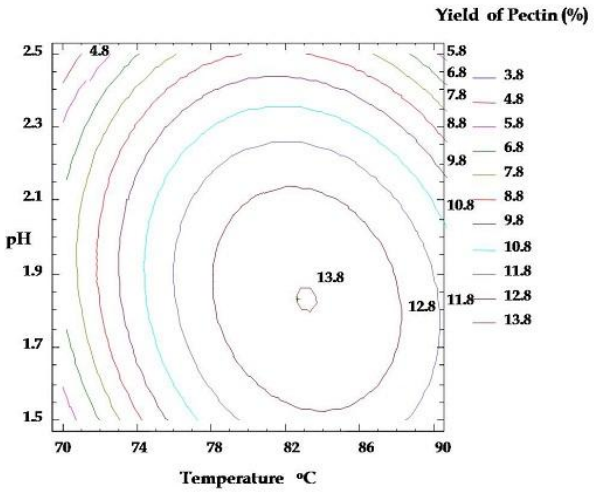


Figure 5. Contours of estimated response surface

In the strong acid, the pectin yield shows a total increasing tendency, for the hydrolysis of pectin is enhanced. However, in too acidic environment, pectin will be over hydrolyzed, decreased, and cracked.

This pane displays the regression equation which has been fitted to the data. The equation of the fitted model is indicated by Equation 2.

The recommended extraction condition is temperature 82.63^{o}C, pH 1.83, time 65 minutes. Carry through pectin extraction under the recommended extraction technology, the yield is 13.781%. Thus the optimization condition is achievable.

4. Conclusions

The independent variable experiments determined the extraction condition is extraction temperature 80^{o} C, extraction time 65 minutes and pH 2.0 under which the pectin

yield is relatively high. RSM was used to determine the optimum condition for pectin extraction from dried pineapple peels on the basis of dependent variable experiment. The dependent variables are extraction temperature and pH together with the response value-yield. By analyzing and preparing the pertinence and significance of every variable, the optimized extraction conditions are temperature 82.63^{o} C, pH 1.83 and time 65 minutes. Carry through pectin extraction under the recommended extraction technology, the yield is 13.781%. Thus the optimization condition is achievable.

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PHYSICOCHEMICAL PROPERTIES OF YELLOW MAIZE-PEANUT FORTIFIED TORTILLAS

Karim Olayinka Ramota[1], Oyeyinka Samson Adeoye[1,2*], Kayode Rowland Monday Ojo[1], Ogunjobi Oluwatosin[1], Ibrahim Alima[1]

[1]Department of Home Economics and Food Science, University of Ilorin, Nigeria
[2]Department of Biotechnology and Food Technology, Durban University of Technology, South Africa
**sartf2001@yahoo.com/+2347066395421*

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ABSTRACT

In this study, maize grain were cooked and soaked in alkaline (nixtamalization). Nixtamalized maize flour was blended with groundnut (5, 10, 15 and 20%). The proximate composition of the flours and tortillas were evaluated. A range of 3.90-5.26, 6.28-14.31, 4.50-16.20, 1.18-1.22, 2.40-2.64 and 61.73-77.44% was observed for moisture, protein, fat, fibers, ash and carbohydrate contents of the nixtamalized maize flours respectively. Upon the addition of groundnut, the protein and fat content increased. Loose bulk density and packed bulk density of the flours varied from 1.45-1.48 g/mL and 1.61-1.75 g/mL respectively. The water absorption capacities (WAC) of the flours increased while oil absorption capacities (OAC) decreased following the addition of groundnut. Calcium (Ca), potassium (K), magnesium (Mg) and phosphorus (P) content of the flours was observed to increase, while the sodium (Na) content reduced after nixtamilization. The proximate composition of the tortillas made from the nixtamalized flours varied from 9.44 to 11.12 for moisture, 5.40-12.96 for protein, 32.10-42.25 for fat, 4.07-6.16 for fibre, 1.53-1.95 for ash and 33.09-43.98% for carbohydrate content. Sensory evaluation of the tortillas showed that all the fortified samples are favorable compared with the control.

1. Introduction

Tortilla is a major staple food for the Mexican people. It is also a popular snack in the United States (Kawas and Moreira, 2001). Tortilla is an important source of protein for the Mexican population, providing approximately 70% of the calorie and 90% of total protein intake (Rosado et al., 2005). Tortillas are made through the alkaline cooking process called nixtamalization. The process consist of cooking corn grain in abundant water and lime and steeping for 8-16 h (Cuevas-Martínez et al., 2010). Steeped corn is milled to obtain masa, which is then used for making tortillas. Nixtamalization process has been reported to enhance the nutritional value of maize by improving protein quality, increasing the calcium content and niacin availability (Cuevas-Martínez et al., 2010). Unfortunately, it also leads to losses of protein, carbohydrates fats and vitamins Milán-Carrillo et al., 2004; Figueroa et al., 2001). Several efforts have been made through research to reduce these losses through improved processing methods. For example an optimized processing method of nixtamalization was recently reported (Gutiérrez-Cortez et al., 2013). High quality tortillas were produced by these authors at a reduced cooking temperature (92ºC) cooking time (25 min) and steeping time (4 h). Further

superior quality products have been made using enzyme nixtamalization (Sahai and Jackson, 2000). Fortification of nixtamalized maize flour using defatted soybean flour (Gonzales-Agramon and Serna-Saldivar, 1988), direct addition of lysine and tryptophan (Waliszewski et al., 2000), spent soy bean residue (Waliszewski et al., 2002), common bean (Cuevas-Martínez et al., 2010), amaranth flour (Vázquez-Rodríguez et al., 2013), rice bran (Al-Okbi et al., 2014), have been reported. However, to the best of our knowledge, no report exists on the use of groundnut in fortifying nixtamalized maize flour for tortilla production. Groundnut is an excellent source of protein, essential amino acids and minerals (Ayoola et al., 2010). Further, with the growing demand of ready to eat snack in many parts of the world, fortifying nixtamalized maize flour with groundnut may provide variety and increase the choice of consumption among many other snacks. Fortification of nixtamalized maize with legumes for tortilla production may influence nutrient composition, texture and sensory properties. For instance, the protein content of tortilla fortified with common bean (*Phaseolus vulgaris*) was reported to increase by approximately 21% (Cuevas-Martínez et al., 2010). Lysine and tryptophan was also found to increase by 53% and 100% respectively following the addition of common bean. Therefore it is important to investigate the influence of groundnut fortification on nixtamalized maize flour. In this study, the proximate composition, essential amino acids, functional properties, mineral composition, of groundnut - fortified nixtamalized maize flours was determined. The proximate composition, texture and sensory evaluation of the tortillas from the fortified flours were also studied.

2. Materials and methods

Yellow maize was obtained from a local market in Benin City, Nigeria. Groundnut was purchased from a market in Ilorin and kept at room temperature. All other reagents (food grade) were obtained from the Food processing laboratory, Department of Home Economics and Food Science, University of Ilorin, Nigeria.

2.1. Alkaline treatment of maize

The optimized method of nixtamilization as described by Gutiérrez-Cortez (2013) was employed in this study Yellow maize was cooked in 1% calcium hydroxide solution (1:3) at 92^{o}C for 25 minutes. The cooked grains were steeped in the cooking vessel for 4 h at room temperature. The cooking solution was drained off and the treated yellow maize was washed with excess water four times with tap water to remove excess calcium hydroxide. After washing, the maize was dried in an oven at 60ºC for 72 h. The dried maize was divided into 5 samples and milled with dried groundnut in ratio 5, 10, 15 and 20% per weight of nixtamalized flour.

2.2. Tortilla production

Tortilla was produced using method described by Salazar (2014) with few modifications. Briefly, nixtamalized flours were reconstituted with enough water in a bowl to produce dough with good consistency. The dough was shaped into cylindrical thin disks (approx. 2mm thickness) using a manual cutter. Frying was done in a deep fat fryer at 190^{o}C until the chips were crispy (approx. 10 mins). Freshly prepared tortilla chips were used for sensory evaluation. Other samples were cooled and kept refrigerated (4^{o}C) until analysis.

2.3. Chemical composition

Proximate composition, amino acid content of flours and tortillas composition were determined according to the official methods of analysis (AOAC, 2000). Carbohydrate was calculated by difference**.**

2.4. Loose and packed bulk density

Flour samples were gently transferred into 10 mL graduated cylinders that were previously weighed. The bottom of the cylinder was gently tapped on a laboratory bench several times until no further diminution of the sample level was observed after it was filled up to the 10 mL

mark. Bulk density was calculated as the ratio of the bulk weight and the volume of the container (Mpotokwane et al., 2008).

2.5. Oil and water absorption capacity

The oil and water absorption capacities of nixtamalized maize flour were determined by the method described by B. Abbey and G. Ibeh (Abbey and Ibeh, 1988). Briefly, one gram of each sample was weighed into a dry, clean centrifuge tube. Sunflower oil (10 mL) with density of 0.98 g/L was poured into the tube and properly mixed by vortexing. The suspension was centrifuged (Ependorf 5810R, Germany) at 3500×g for 20 min. Supernatant was discarded and the tube with its content reweighed. Gain in weight expressed, as a percentage of oil bound, was calculated as the OAC of the sample. The same procedure was repeated for WAC by replacing oil with water.

2.6. Mineral composition of flours

Flours were digested as described by (Amonsou et al., 2011) and the mineral content of the digest was determined by methods of AOAC (2000).

2.7. Sensory evaluation

Panel members were selected from students and lecturers within the Faculty of Agriculture University of Ilorin (both sexes, 22 to 45 years old). The panel evaluated the products for their sensory qualities (taste, colour, odour, texture and overall acceptability) using a nine-point hedonic scale. 1 and 9 represents dislike extremely and like extremely respectively. Analysis of variance (ANOVA) was performed on the data gathered to determine the significant difference in the samples. The judges were made to wash their mouth with water after evaluating each product. The selection criteria for the panelists were based on the participant interest, taste, colour, aroma, texture perception and overall acceptability were determined, besides that they all declared to enjoy eating tortillas and to consume them on a regular basis.

2.8. Statistical data analysis

All analyses with mean and standard deviations were determined in duplicates. Data were analyzed using the Analysis of Variance (ANOVA) statistical method (Statistical Analysis System version 9.2 program, SAS Inc., (2012), USA.). Means were separated using Duncan's multiple range test. Significant differences were established at $p \leq 0.05$.

3. Results and discussions

3.1. Proximate composition of groundnut fortified-nixtamalized maize flours

The proximate composition of groundnut fortified-nixtamalized maize flours is shown in Table 1. The protein content of maize flour decreased (approx. 23%) after nixtamalization process (Table 1). Similar reduction in protein content of maize flour following nixtamalization have been reported (Cuevas-Martínez et al., 2010; Milán-Carrillo et al., 2004). However, there was an increase in the protein content following the addition of groundnut. Nixtamalized maize flour (NMF) fortified with 20% groundnut had the highest protein content (14.31%). The protein content of the groundnut-fortified NMF were slightly higher than those reported for nixtamalized maize flour fortified with common bean (Cuevas-Martínez, 2010), and those fortified with bean and amaranth (Gutiérrez-Cortez et al., 2013). Nixtamalization also decreased the fat content of the maize flour. However, the decrease was very minimal (approx. 9%). The decrease in fat content after nixtamalization may be attributed to losses from the pericarp and germ tissues during cooking and washing (Milán-Carrillo et al., 2004); Serna-Saldivar et al., 1990). Moisture, fibre, ash and carbohydrate contents were not significantly affected by nixtamilization.

3.2. Essential amino acids of fortified-nixtamalized maize flours

Leucine was the major essential amino acid (EAA) present in the untreated maize flour (Table 2). The EAA composition of the untreated maize flour is within values reported

in the literature (Andresen et al., 1998). All the EAA was found to decrease after nixtamilization. The highest and lowest decrease was observed for tryptophan (approx. 41%) and lysine (approx. 2%) respectively. A similar decrease in the tryptophan content of nixtamalized maize flour has been reported (Cuevas-Martínez et al., 2010); Rojas-Molina et al., 2008). However, these authors reported approximately 39% and 53% decrease in tryptophan content respectively. The short soaking time (4 h) and low temperature (92^{o}C) employed in this present study may have accounted for the variation observed in comparison with previous findings. According to Rojas-Molina (2008), protein solubility distribution and amino acid localization during nixtamalization may have accounted for the changes in protein quality as reflected by a decrease in the EAA. Upon the addition of groundnut to the nixtamalized maize flour, however, an increase in the EAA was observed. The increase in amino acids may be attributed to the complementary effect of proteins in the groundnut. Similar increase in amino acid content of maize flour after nixtamalization has been reported (Cuevas-Martínez et al., 2010).

3.3. Functional properties of the maize flours

The loose bulk density (LBD) and packed bulk density (PBD) of the flours were not affected by nixtamalization (Table 3). Although there was a slight increase in LBD and PBD following the addition of groundnut, the increase was not significant. Similarly, there was a slight increase in the water absorption capacity (WAC) of the flours after nixtamalization. However, the observed WAC were very much similar among the flours. The increase in the protein content of the fortified flours (Table 1) may have accounted for the slight increase in WAC. This seems plausible since proteins have been reported to contribute to WAC of food materials (Ravi and Susheelamma, 2005). On the contrary, the oil absorption capacity (OAC) of the flours decreased following nixtamalization. Further, the OAC also decreased with increase in the addition of groundnut flour. This may be due to the relatively high fat content in the flours (Table 1) after groundnut addition. The increase in fat content in the flours may have prevented the flours from absorbing more oil. This may be advantageous in tortilla preparation as the tortilla produced from the fortified nixtamalzed maize flour absorbed less oil compared to unfortified samples (Table 5). Similar increase in the WAC of nixtamilized maize following the addition of cowpea has been reported (Afoakwa et al., 2007).

3.4. Mineral composition of the flours

The calcium (Ca), potassium (K), magnesium (Mg) and phosphorus (P) content of the flours was observed to increase after nixtamilization (Table 4). However, the sodium (Na) content reduced for all the flours. The initial increase in the Ca content of the flours prior to the addition of groundnut may be attributed to the absorption of Ca from the lime soaking process by the maize grains. According to Cuevas-Martínez (2010), nixtamilization increases the Ca content of maize flours. Further, calcium ions are reported to be carried by water through the tip cap, germ and pericarp and are mostly retained in the germ (Serna-Saldivar et al., 1990). However, the calcium content of nixtamalized maize flour has been found to depend on the concentration of the lime used, cooking and steeping time (Serna-Saldivar et al., 1991). The further increase in the Ca, K, Mg and P contents of the flours may be attributed to the added groundnut. Groundnut has been reported to be a good source of Ca, K, Mg and P (Ayoola and Adeyeye, 2010).

3.5. Proximate composition of tortillas

The protein content of the tortillas reduced slightly after frying when compared to the flours (Table 5). This may be attributed to the effect of thermal treatment on nutrients. Tortillas without groundnut addition had the highest reduction in protein content (14%). However, the fortified tortillas had relatively higher protein content than the control. The

protein contents of the tortillas are comparable to those reported for tortillas fortified with common bean (Cuevas-Martínez et al., 2010) and amaranth (Vázquez-Rodríguez et al., 2013).The fat content of the fortified tortilla samples were higher than the control. There was an increase in the fat content compared to the flours. However, tortilla fortified with 5% groundnut (MTG_5) had the highest fat content (42.25%). The higher tendency to absorb more oil as shown by the OAC (Table 2) of this flour may have accounted for the higher fat content of the tortilla. Beyond 5% level of groundnut addition, the fat content of the tortillas were similar.

The moisture, ash, fibre and carbohydrate content of the tortillas were not very much different. The moisture content (9.44-11.12%) of the tortillas in this study were considerably lower than those reported for tortilla fortified with common bean (Cuevas-Martínez et al., 2010). These authors reported moisture content in a range of 47.11 to 48.16%.

Table 1. Proximate of the groundnut fortified nixtamalized maize flour (g/100g)

Sample	Moisture	Protein	Fat	Fibre	Ash	CHO
A	4.36^{ab}±0.19	8.12^{b}±0.98	4.50^{ab}±0.32	1.18^{a}±0.00	2.40^{a}±0.01	79.44^{cd}±0.64
B	5.26^{c}±0.34	6.28^{a} ±0.11	4.08^{a}±0.32	1.19^{ab}±0.00	2.51^{a}±0.00	80.68^{d}±0.55
C	4.08^{a}±0.03	10.34^{c}±0.26	10.65^{c}±0.49	1.19^{bc}±0.00	2.54^{a}±0.01	71.20^{c}±0.25
D	4.38^{ab}±0.88	10.72^{c}±0.16	13.00^{d}±0.00	1.19^{bc}±0.00	2.57^{b}±0.00	68.14^{b}±0.32
E	4.79^{ab}±0.27	12.80^{d}±0.09	14.50^{e}±0.42	1.20^{c}±0.01	2.60^{b}±0.01	64.11^{ab}±0.59
F	3.90^{a}±0.43	14.31^{e}±0.26	16.20^{f}±0.49	1.22^{d}±0.00	2.64^{b}±0.00	61.73^{a}±0.33

Mean±SD. Means with the same superscript are not significantly different ($p \leq 0.05$).
CHO: Carbohydrate (calculated by difference)

Table 2. Essential amino acids of nixtamalized maize-groundnut blend (g/100 g)

Sample	Lys	Try	Leu	Iso	Met	Phe	Val	Thr	His
A	3.92	1.92	6.22	3.68	1.92	3.39	4.89	3.92	2.34
B	3.82	1.14	5.43	3.35	1.68	3.09	4.69	3.78	1.72
C	4.46	1.19	8.67	3.42	2.07	3.99	4.90	4.02	2.22
D	4.51	1.25	7.80	3.84	2.12	4.47	5.39	4.09	2.08
E	4.60	1.22	7.90	3.96	2.24	4.83	6.39	5.11	2.11
F	7.68	1.28	8.00	4.01	2.89	5.01	6.53	5.01	2.10

Table 3. Functional properties of nixtamalized maize-groundnut blend

Sample	LBD (g/mL)	PBD (g/mL)	WAC (%)	OAC (%)
A	1.45^{a}±0.01	1.61^{a}±0.04	180^{a}±0.42	215^{c}±0.46
B	1.46^{a}±0.02	1.62^{a}±0.01	205^{a}±0.64	192^{bc}±0.01
C	1.48^{ab}±0.01	1.64^{a}±0.01	240^{a}±0.28	146^{abc}±0.25
D	1.49^{ab}±0.00	1.65^{a}±0.03	235^{a}±0.07	128^{ab}±0.13
E	1.48^{ab}±0.01	1.68^{a}±0.01	230^{a}±0.14	101^{a}±0.52
F	1.48^{ab}±0.00	1.75^{b}±0.01	225^{a}±0.07	97^{a}±0.33

Mean±SD. Means with the same superscript are not significantly different ($p \leq 0.05$).

Table 4. Mineral composition of nixtamalized maize-groundnut blend (mg/g flour)

Sample	Calcium	Magnesium	Sodium	Potassium	Phosphorus
A	169.74	94.87	749.14	984.18	462.72
B	342.19	144.14	531.62	1024.49	584.41
C	356.99	183.19	468.44	1113.13	638.86
D	368.37	218.89	472.31	1126.36	612.91
E	383.41	217.94	475.37	1129.46	610.75
F	386.39	217.97	476.39	1129.52	609.24

Table 5. Proximate composition of tortillas (g/100g)

Sample	Moisture	Ash	Protein	Fat	Fibre	CHO
MTG_0	11.12^{b}±0.5	1.60^{b}±0.10	5.40^{a}±0.10	32.10^{a}±1.10	5.80^{b}±0.01	43.98^{b}±0.12
MTG_5	9.58^{a}±0.19	1.60^{b}±0.00	9.41^{b}±0.12	42.25^{c}±0.09	4.07^{a}±0.02	33.09^{a}±0.10
MTG_{10}	9.44^{a}±0.23	1.85^{c}±0.05	9.77^{b}±0.41	38.40^{bc}±0.40	4.47^{a}±0.02	36.07^{a}±0.14
MTG_{15}	9.90^{a}±0.05	1.95^{c}±0.00	11.84^{c}±0.11	35.83^{a}±0.09	5.75^{b}±0.05	34.73^{a}±0.15
MTG_{20}	9.63^{a}±0.19	1.53^{b}±0.03	12.96^{c}±0.22	34.40^{a}±0.21	6.16^{c}±0.01	34.32^{a}±0.32

Mean with the same superscript are not significantly different ($p \leq 0.05$).
CHO: Carbohydrate (calculated by difference)
MTG_0= Maize tortilla with no groundnut
MTG_5= Maize tortilla fortified with 5% groundnut
MTG_{10}= Maize tortilla fortified with 10% groundnut
MTG_{15}= Maize tortilla fortified with 15% groundnut

Table 6. Mean sensory scores for tortillas

Sample	Taste	Colour	Aroma	Texture	Overall acceptability
MTG_0	5.80^{a}	6.55^{a}	5.65^{a}	6.25^{a}	6.30^{a}
MTG_5	6.85^{ab}	7.00^{a}	6.20^{a}	6.70^{a}	6.80^{a}
MTG_{10}	7.20^{b}	6.65^{a}	6.15^{a}	6.55^{a}	6.75^{a}
MTG_{15}	6.65^{ab}	6.00^{a}	6.10^{a}	6.45^{a}	6.60^{a}
MTG_{20}	6.45^{ab}	6.30^{a}	6.20^{a}	6.05^{a}	6.25^{a}

Means with the same superscript are not significantly different ($p \leq 0.05$).

The drying temperature and duration of the nixtamailized maize flour may have accounted for the difference. In this study the nixtamailized maize flour was dried prior to tortilla production. The lower moisture content of the tortillas may indicate better keeping quality.

3.6. Sensory evaluation of tortillas

The mean sensory scores of the tortillas are very much similar (Table 6). The ratings for taste colour, aroma texture and overall acceptability showed that the incorporation of groundnut to nixtamalized maize flours had no significant effect on the tortillas. Although, tortillas made form nixtamalized maize flour fortified with 5% groundnut had the highest rating for overall acceptability, the ratings compared to other tortilla samples were very much similar. This suggests that the inclusion of groundnut to nixtamalized maize flours did not significantly alter the sensory attributes of tortilla.

4. Conclusions

Fortification of nixtamalized maize flour produced acceptable tortillas.

There was an improvement in the protein and fat content of the nixtamalized maize flours. Amino acid content also improved following the addition of groundnut. The flours exhibited good oil absorption capacity which moderated the absorption of oil during frying. Up to 20% addition of groundnut flour did not affect the sensory properties of the tortillas. Fortification of nixtamalized maize flour with

groundnut should therefore be encouraged. Studies on in-vitro digestibility and sorption isotherms of the groundnut fortified tortillas may be investigated. The latter may give useful information in the design of packaging materials for the product.

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FUNCTIONAL THEORY IN TRANSLATING CHINESE TRADITIONAL FOOD MANUAL

Yi Dang *

*Foreign Language Institute of North West University; *dangyidyd@163.com*

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ABSTRACT

Functional theory works as an important breakthrough and supplement for traditional equivalence theory. It also provides a new sight for translating non-literary works. Food product instructions (manual) are a kind of practical writing with its own style and genre. Focusing on the translation of Chinese traditional food product instructions, this paper analyzed its content and feature, and discussed the two specific translation strategies, foreignization and domestication, which make the translation process more flexible and effective so as to satisfy the expectation of translation.

1. Introduction

With the development of economic globalization, there are increasingly more enterprises applying bilingual instructions. So nowadays, product instructions with both English and Chinese are around everywhere. For food is a necessity in daily lie, the translation of its instruction not only determines the approval and acceptance from foreign consumers, but also leaves great benefits on the sales volume of products. On contrary, instruction translation with poor quality will give a bad feeling to consumers and even reduce the whole sales volume (Yi and Mei, 2011). In recent years, the public are increasingly more aware of the importance of food product instructions translation. Besides, more enterprises begin to attach great importance on translating food product instructions. It is one of urgent problems facing the translation field to establish relative translation principles and strategies available for operation for some guidance on the translation practice in a general and effective way. There are lacks of studies on food packages English translation. Moreover, those existing relative studies simply focus on a small amount of translation methods or mistakes. There are lacks of scientific integration but too much one-sidedness (Xiaoliang and Jirong, 2012). This paper analyzed different texts according to different purposes as a whole based on the functional translation theory, and then pointed out translation theories according to different purposes. In this way, it can not only provide appropriate theory basis to food package English translation, but enable studies in this field to be an orderly regulated system (Haiyan, 2014). Functional translation theory emphasizes that translation is a kind of special communication, involving three texts: the original text, the schema text and the translation (Bing, 2012). In terms of the original text, what is the most important is to understand the rhetoric functions of authors. An accurate understanding of rhetoric functions of source language is the key to the ideal schema text. Moreover, an accurate idea of original cognition schema is the basis for understanding rhetoric functions of original text correctly. The ideal schema text comes from the cognition schema of original text and the accurate

understanding of rhetoric intentions of original writers. Based on this schema text, translation text of target language should not only consider the equivalency of rhetoric functions, but take the translation purpose and readers into account. This paper made a brief analysis and study on the English translation of Chinese traditional food manual based on functional theory.

2. Materials and methods

2.1 Content and feature of functional theory

When Nida's equivalency theory and translation theory from linguistics prevailed, Katherina Reiss, as a leader in German functional theory, first considered the special purpose which translation should reach as a new model of translation criticism. She divided the text functions into three categories: information, expression and inducement. In many cases, a text may contain double or multiple functions, which means that it is supposed to combine different translation strategies during the translating process (Haidan, 2014).

Afterwards, her student Hans J. Vermee put forward the Skopos theorie, which turned the most fundamental content of translation functional theory since its appearance. It introduced the "Act Theory" into the translation theory, and then regarded translation as a cross-culture communication with purpose. The purpose that translation should reach determines the strategies that translation should adopt, namely purpose determines means, and this is the prime principle of Skopos theorie - Skopos rule (Ruyu, Zhengqin and Han,). There are other two rules in Skopos theorie, including the coherence and fidelity. The former means that the translation must conform to the target language, and the latter means that the translation should not violate the original text. These two rules are dominated by the Skopos rule.

Different from the traditional equivalency theory, what the functional theory emphasizes is not whether the translation equals to the original text or the translation is perfect, but translation should aim at the expected functions based on analyzing the original text, and select the best way to handle according to all factors about context. Compared to the traditional translation methods on the basis of equivalency theory or the extreme functionalism, this obviously has a great improvement and appears to be quite scientific and available for operation. Besides, it provides a sound and objective basis for translation, a kind of cross-culture communication.

Functional theory considers the objective as the general principle and it studies the translation with the act theory and cross-culture communication, which provides a theoretical evidence for translators to flexibly handle the target texts and diversify the translation standards. In terms of those texts with a clear purpose, such as instructions, advertisements, trademark and film title, it especially offers some guidance on the translation practice and its strategies.

Food product instructions is a kind of practical writing with a clear purpose and practicability, Skopos theorie as the fundamental theory of functional translation, can be applied in guiding the translation of food product instructions. Both traditional" faithfulness, expressiveness, elegance" in China and Nida's equivalency theory lose sight of the purpose of original text. By contrast, Skopos theorie provides the translation of food product instructions with a new sight, which enables the translating process more flexible and effective.

2.2 Content of food product instructions and the translation status

Food product instructions are mainly the instructions on food packages, and it contains the following aspects: name, ingredients, exterior looks, flavor, nutrient facts, suit crowds, handling information and storage. For food product an instruction mainly appears on the food packages with limited space, it features clear and concise. By referring to many foods product instructions, it is easy to find a great many of Chinese tradition four-

character idioms from the content, such as "口感清新，风味独特，香浓幼滑". There are full of culture factors within the Chinese traditional food product instructions, such as Ma Po bean curd, Woyang Tai Gan, and dried ballonflower. Food products instructions help to bridge the gap between consumers and products. In terms of consumers, manuals enable them understand and handle products, so as to avoid some mistakes from misunderstanding. It enjoys three main functions, (1) information: provide specific information about the feature and usage; (2) aesthetic: enable readers a pleasure in sense from the description about food products; (3) imperative: make readers make expected response. These features and functions of food product instructions determine its flexibility and complexity in translation (Jia, 2012).

Food product instructions are attracting the attention of more and more enterprises, while there are too many errors in its translation. Main errors include the transliteration, such as translate "陈皮, 甘草" into "chenpi, gancao"; errors in pinyin, such as translate "名师制作" into excellent cooker; and Chinglish, such ad translate "拳头产品" into "fist product". Besides, there are also some other problems, like culture misunderstanding. Solutions of these problems are in urgent need of suitable translation strategies for guidance.

3. Result and discussion

Food product instructions not only need to introduce product instructions to consumers, provide them with understanding, but also arouse their desire of purchase and sell their products. According to the three types of texts divided by Reiss, the translation of manual is not only concise and common, but also of strong persuasiveness and charm, which makes consumers feel pleased to understand and accept. Therefore, in translating process, we should integrate several translation strategies and methods to produce better effects.

3.1 Apply foreignization in handling the expression form of text information

"Foreignization requires translator to be more creative, and present the content, style and feature of the original text in a faithful and complete way, without totally abiding by the original literal expression. Foreignization is special because it presents the essential content of original texts in precise language and draws upon the essence while discarding the exterior form (Wei, Mi and Qiaobin, 2013). For there is a great difference in style of food product instructions in Chinese and English, it is needless sticking to the content and expression form of original text when translating. What is more important, we should give full play to the creativity of translators so as to help the translation more conform to the rules of English grammar (Liang, 2013). In order to reach the expected goal of translation, amplification, omission and restructuring can be applied in translating process.

(1) Original text：即购即食，食用方便。(小米锅巴)

Translation: (Always) ready to serve.

If it was literally translated into "Opening and eating immediately", foreign consumers are likely to doubt whether food will turn bad without using up. This kind of translation undoubtedly will leave a quite bad impression on others. We should apply the rule of foreignization and turn it into "ready to serve" according to English habits. In this way, foreign consumers will understand that they can enjoy this product any time without extra processing.

(2) Original text：本品纯属天然，食之香醇幼滑，营养丰富全面，老少皆宜，即冲即食，食用更方便。

Translation: It tastes pure, delicate and smooth with rich nutrition. It is a convenient and healthy food for you.

Translation bravely applies the simple and concise English words in place of Chinese four-character idioms in original text. The whole

sentence reads quite coherent and clear, and is easy to be accepted and understood by foreign consumers. So it reaches the purpose of food instruction.

3.2 Apply domestication when handling cultural factors

Food as a unique product from one country is always branded with culture, while the simple and concise food product instruction often throws the cultural factors out of consideration. However, different from literature works, its fundamental purpose is not to carry forward the culture, but to express some information and realize communication. So as to give foreign consumers a full understanding and then purchase some certain Chinese traditional food, it should apply domestication when handling cultural factors in food product instructions, or we cannot reach the purpose of promoting our products (Lei and Yu, 2013).

(1) Original text: 金丝小枣(土特产)

Translation: Honey-sweet Dates

If we translated "金丝小枣" into "Gold Silk Small Dates"according to foreignization, it would seem that every vocabulary corresponds to the Chinese name, but in fact it is a kind of misleading. English and Chinese nations are different in way of thinking for the same thing. If we left this behind, it should lead to a pragmatics mistake in translation and hinder the information communication. Foreign consumers will imagine that this product has cocoon fibers and is small in size when reading "Gold Silk Small Dates", while losing sight of its excellent quality---sweet and tasty. The adaption masters the main feature of product, namely Honey-sweet, which is able to attract the purchase desire of English readers.

(2) Original text: "产自云南高山云雾之中，清明前精心采制……"

Translation: Premium tea prepared with leaves picked from Yunnan Mountain at the right time around April.

If we translated "清明前" into "before the Tomb-sweeping day', although domestic consumers knows that tea will be of better quality if picked around the Tomb-sweeping day, for a lack of related cultural background, foreign consumers would find it hard to relate the quality of tea to the right moment of picking tea, let alone the relation between the pleasant smell and cold tomb. So as to reach the communication purpose of food product instructions, we can apply domestication which centers on readers and adapt it into "at the right time around April". In this way, the translation becomes more simple and easier to be accepted and understood by foreign consumers.

(3) Original text: 待油烧热(但切忌冒烟)然后将干虾片放入油锅翻炸片刻。

浮起后捞出，则成松脆的熟虾片。

Translation:Heat until it boils. Deep –fry prawn crackers.

Wait until it floats and has expanded to its full fried size. Drain oil.

When ensuring the information is correctly and completely conveyed, translation should conform to the habits of consumers, such as language using, cultural and consumer physiology (Qing and Xiuli, 2013). Therefore, we should apply domestication in transforming the translation into a form familiar to consumers. In terms of cultural habits, in order to eliminate differences and instructions in cultural cognition, we should apply domestication to make it close to the cognition habits of consumers. Delete the information "但切记冒烟" in original text and directly translate into "Heat until it boils". In consumer's culture, cooking smoke in kitchen remains a taboo. Generally the next step is performed when the oil boils. If we just copied the original text mechanically, it would seem too necessary. "翻炸片刻" in original text gives consumers a vague idea of the cooking time, which does not conform to the cooking habits. Here it is translated into "Deep-fry". Although there is still no specific cooking time,

it is required to be "deep" in degree with a clearer instruction. The prawn crackers in original text are simply required to "浮起后捞出", but there are amplifications of "expanded to its full fried size" and "Drain oil". These give a more precise and vivid description about the prawn crackers in frying degree and requirement out of oil, which meet the consumers' expectation for this kind of information.

These above examples show that we should not only take account of the expression form and cultural connotations of food instructions, but the cultural features and acceptance of target consumers in the English translation practice of food product instructions. Based on different needs, we should combine together the two strategies, foreignization and domestication for flexible application under the guidance of Skopos theorie to reach a better translation and realize the expected purpose of food translation.

4. Conclusions

Translation functional theory centers on the communication purpose of translation and plays an emphasis on the practicability of text. Therefore, compared to other translation theory, it can provide better guidance on translating texts with strong practicability such as the English translation of Chinese traditional food manual in this paper. When translators translate the food product instructions based on functional theory, they not only should have a full understanding of the difference in language and culture between China and the west, take account of the cross-cultural communication purpose in original text, but should make some effective translation strategies and skills flexibly to enable the full realization of expected purpose of original text.

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Food Technology Student Contest - COSTA 2014

Carpathian Journal of Food Science and Technology (Chemistry-Biology Department of North Universitary Center of Baia Mare, Technical University of Cluj-Napoca, Romania) organized the 5th edition of Food Technology Student Contest-COSTA 2014 (CONCURSUL STUDENȚESC DE TEHNOLOGIE ALIMENTARĂ – COSTA 2014), in 11.12.2014.

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- To build a platform of professional communication between future graduates and food industry business.
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Event organisers:

Professor dr. Anca Mihaly Cozmuta ancamihalycozmuta@gmail.com

Lecturer dr. Anca Dumuta codre_anca@yahoo.com

Lecturer dr. Liviu Giurgiulescu giurgiulescu@gmail.com

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