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SAVOURY FLAVOUR FORMULATIONS FROM MAILLARD REACTION OF HYDROLYSATED COD BY-PRODUCTS AND SUGARS

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ABSTRACT

On a world scale, more than 20 million tons of the fish production is turned into non-food products. These by-products contain proteins and fish oils that can serve as a raw material for valuable products. Hydrolysis, autolytic or by enzymes, is one way for protein recovery. In South-East Asia fish sauces are an example of auto-hydrolysed products having strong and enhancing fish flavour. The aim of this study was to test if fish by-products could be used as a source of savoury reaction fish flavours. In this study a fish protein hydrolysate (FPH) was produced by enzymatic hydrolysis of minced cod cut-offs (CCHs). The resulting FPH was heated in the presence of sugars to produce reaction fish flavours (fish sauce analog) in the Maillard reaction (MR). Low degree of hydrolysis (DH) (38.6%) of CCHs were obtained by the combination of 1% of Protamex® and 1% of Flavourzyme® however approximately 70% of the proteins were found in the clear supernatant. After heating the FPH in the presence of 1% sugars, xylose and lactose enhanced the fish sauce-like flavour but glucose, ribose and fructose less. The Maillard reaction products (MRPs) from xylose heated for more than 1.5 hours at 80°C gave the characteristic fish sauce flavour but the lactose mixture had a typical and strong fishy flavour. With increasing reaction time, the fishy flavour decreased while the burnt flavour was enhanced. The optimal conditions of the parameters (xylose concentration, reaction temperature and reaction time) for colour production were determined by RSM targeting also optimal fishy and burnt flavour tones. Based on the results from the RSM a fish sauce analog (FSA) was produced using 1.5% of xylose heated together with FPH at 90 ℃ for 0.93 hours. The FSA had a deep brown slightly reddish-yellow colour having strong fishy and burned flavour. The protein content was 13.6% and it hat 3.0% salt. It is concluded that FPH made from cod-cut offs can be used as a raw material for the production of fish reaction flavours. The product could be marketed as a low salt alternative in SA-Asia and elsewhere where fish sauces are used in food formulations.

Keywords: flavour, cod, by-product, Maillard reaction, response surface methodology, fish sauce.

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1 INTRODUCTION

1.1 Background

Traditionally, fish by-product from the fish processing factories such as fillets cut-off and viscera etc. are produced into fish meal and animal feed. In 2008, nearly 14 percent (20.8 million tons) of world fish production destined for non-food purposes was reduced to fishmeal and fish oil (FAO, 2010). The protein contents of fish by-products are almost as high as that of fillets (Table 1) (Bechtel, 2003). However, recent years, raised awareness on the profits loss from fish by-products has guided the fish processing enterprises and researchers making concerted efforts to make the best use of all raw materials, enhance the added value and pursuit value maximization. Meanwhile, the aquatic product processing wastes which process additional biological waste pollute the environment. Nowadays, more and more research works focus on the utilization of fish by-product as raw materials or ingredients for improving additional valve of product (Bhaskar N., 2008; Cha *et al*., 2003; Cai *et al.*, 2016; Gildberg *et al.*, 2002; Gálvez A, 1985; Ovissipour *et al.*, 2012).

Table 1: Protein contents in different parts of three species fish

	Alaskans Pollock (%)	Pacific Cod
Head	15.2	16.4
Viscera	15.2	13.0
Frame	16.3	15.8
Fillets	17.5	18.2

Fish by-products have been utilised for the production of fish protein hydrolysates (FPH), used as nitrogen source for microbial growth. Autolytic process, which depends only on endogenous enzymes, is considered to be economically advantageous; however, exogenous commercial enzymes are preferred since they can control hydrolysis, hence control over the quality of the final products. Several enzymes have been frequently utilized for the hydrolysis of fish proteins (e.g., Papain, Alcalase, Neutrase, Flavourzyme, and Protamex) (Bhaskar N., 2008; Chung *et al.*, 2006). Characteristics of the final hydrolysates will depend on enzyme type, the enzyme(s) added, time of enzymatic hydrolysis reaction, additive added and the substrate which plays an important role in the hydrolysis (Kouakou *et al.*, 2014). The enzymatic degradation not only increases the amount of amino acids and low molecular weight peptides that possess unique taste properties, including sweet, salty, sour, bitter, and umami tastes, but also generate volatile and the volatile compounds, which can enhance the flavour of protein hydrolysates (Normah, *et al.*, 2004; Slizyte *et al.*, 2005; Liaset *et al.*, 2000).

Flavour is an important factor to evaluate the quality of fish and aquatic products as well as consumer acceptance (Rehbein & Oehlenschläger, 2009). This characteristic aroma is influenced by the species but also by the conditions used for its post-harvest handling, storage and cooking. Some fish such as salmon or trout, have a strong flavour while might have a relatively mild smell before cooking that becomes strong and pleasant after heating (Berger, 2007). Important aroma compounds which is characteristic of fresh fish, are volatile compounds mainly generated from lipid by oxidative enzymatic reactions and autoxidation of lipids such as aldehydes and ketones. However, compounds derived from Maillard reaction such as pyrazines and furans, also make important contributions to the flavour and aroma of fish products after frying or grilling (Eric *et al.*, 2013; Gálvez A, 1985).

Maillard reaction technology is commonly used by the flavour industry to produce the similar aroma and taste properties to thermally treated foodstuffs such as meat, chocolate, coffee, caramel, popcorn or bread (Taylor & Linforth, 2010). Some research works have been done in the fields of fish by-product hydrolysis reaction and the flavours generated by the Maillard reaction (Kouakou *et al.*, 2014; Ogasawara *et al.*, 2006), while researches of adding additives during the Maillard reaction to enhance the Chinese traditional flavours were seldom reported.

Traditional Chinese foods are Yu-lu, oyster sauce, soy sauce and soybean paste. The main body flavour of which is umami, salted and kokumi. China has a large population, and with the influences of immigration and tourism popularity, Chinese ingredients and restaurants are widely distributing in the world (Nachay, 2015). Therefore, the market of Chinese traditional flavour products has broad prospects and enormous development potential.

Cod is the most popular fish and Cod fillets is one of the traditional processing methods in Iceland (Table 2) (Statistics Iceland, 2015) . Large quantities of cut-offs are left by the processing of cod fillet, and it make possible that the cut-offs could become the sufficient raw material for Chinese traditional flavour products. Meanwhile it will provide significant profits for Icelandic enterprises. It is practical that the Icelandic cod cut-offs could be utilized as raw material developing Chinese traditional flavour products to meet Chinese market.

Table 2: Catch by species and place of landing (January 2000 - November 2015).

Year	2014	2013	2012	2011	2010	2009
Cod (tons)	239,950,585	236,303,429	204,955,148	182,353,125	178,597,230	188,989,054

The research of the project can also provide a mode referred to utilize fish by-products from increasing Chinese fisheries processing such as head, frame, viscera and skin etc. especially for the fresh water fish by-products (Figure 1).

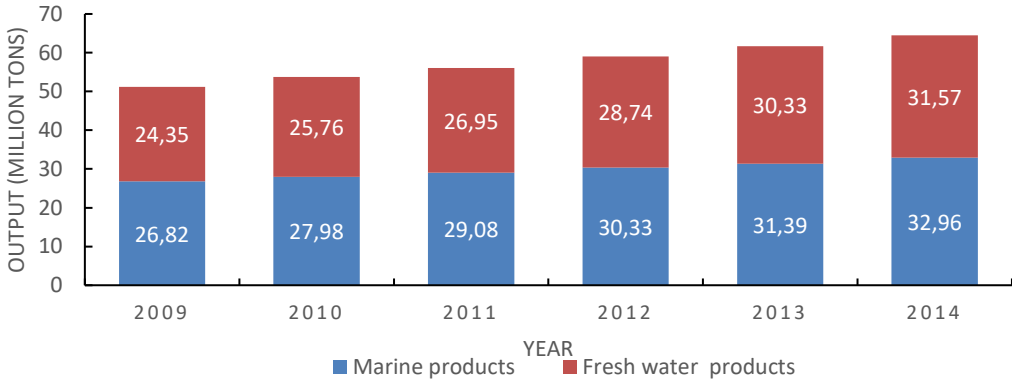

Figure 1: 2009-2014 Chinese output of aquatic products

Salt could not only extend shelf life, but also improve the taste of the product. However, the intake high quantity of salt has the potential risk of high blood pressure. Therefore, the final product can be alternative of fish sauce for healthy condiment and welcomed by the market.

1.2 Goal

The goal of this project was to utilize by-products from Icelandic fish industry to generate seafood flavour formulations after enzymatic hydrolysis of cod cut-offs and followed by heating in the presence of additives (sugars), and then to develop a product of Chinese traditional seasoning character (fish sauce analog). The project has four main tasks with the following objectives:

1.3 Objectives

- To produce hydrolysates of cod cut-offs with high degree of hydrolysis (DH) on the optimal conditions and clear appearance.
- To use the CCHs as an ingredient for generation of seafood flavour(s) with the aid of sugars.
- To obtain fitting equation of the parameters and the Maillard reaction between xylose and CCHs.
- To develop the product with Chinese tradition flavour with market competitiveness.

1.4 Highlights

- Proteases were used to obtain amino-acid-rich ingredients from cod cut-offs.
- Sugars enhanced the flavour of FPH via the Maillard reaction.
- Response surface methodology (RSM) was applied to study the processing parameters effecting on the Maillard reaction between xylose and FPH.
- Low-salt fish sauce analog was developed with a tasteful and umami flavour.

2 LITERATURE REVIEW

2.1 Fish enzymatic hydrolysis conditions

Compared with the chemical process, using enzyme to produce fish by-products hydrolysates has advantages of mild reaction conditions with lower temperature, lower pressure and a pH range between 5 and 8, which could obtain the functional bioactivities such as antioxidant, antihypertensive, antimicrobial, antidepressant, antithrombotic and immunomodulatory activities (Šližytė, et al., 2009; Himaya *et al.*, 2012; Jense *et al.,* 2014). Enzymatic hydrolysis is also the effective method to treat with fish by-products protein. Catla (*Catla catla*), an Indian freshwater major carp, visceral waste hydrolysate was prepared by multifect®-neutral (Bhaskar N., 2008). Yellowfin tuna (*Thunnus albacares*) viscera hydrolysate was produced by using Alcalase 2.4 L (Ovissipour *et al.*, 2012).

Several factors cooperatively influence the enzymatic hydrolysis reaction, such as the type of enzyme, the ratio of enzyme to substrate, pH, time, and temperature, that make the industrial processes more controllable (Liaset *et al.*, 2000). Different proteases were researched to hydrolyse various fish by-products. For the higher yield and protein recoveries, salmon frames were hydrolysed by alcalase and that cod frames were handled with pepsin compared with the other two proteases (Liaset *et al.*, 2000). Compared with the other four enzymes, Alcalase, Neutrase, pig and cod trypsins, Protamex hydrolysates of cod backbone mince had the best recoveries of protein (Gildberg *et al.*, 2002).

It is difficult to utilize the same enzymatic reaction conditions meeting the demand of various aspects (Slizyte *et al.*, 2005). Therefore, the aim, that is, desirable quality indicators for research work or industrial manufacture, should be determined before it is designed, such as the highest of the degree of hydrolysis (DH), protein recovery, the yield or the biological activity. The indictors of DH and the biological activity are frequently focused on in the research work, while the yield is what the manufactories mostly care about. To obtain a higher degree of hydrolysis, the optimum conditions of Catla viscera hydrolysis were an enzyme to substrate level of 1.25 % (v/w), temperature of 55 ℃ and a hydrolysis time of 165 min (Bhaskar N., 2008). The optimum conditions of Yellowfin tuna (*Thunnus albacares)* viscera hydrolysis were: 60.4 °C, 90.25 min (Ovissipour *et al.*, 2012). Slizyte etc. discussed the optimum conditions for the maximum FPH and sludge yields of cod viscera (Slizyte *et al.*, 2005).

2.2 Maillard reaction

Maillard reaction is one of the most important food chemical reaction to produce delicious flavour by carbonyl of reducing sugars and amino group of amino acid or peptide. It can produce special flavour in roasted meat and baked bread (Damodaran *et al.*, 2007).

Recently, some researches were interested in the bioactivities of Maillard reaction products (MRPs) of protein hydrolysates. The MRPs of deboned chicken residue hydrolysates had the strong antioxidant properties of reducing 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and inhibiting lipase (Sun *et al.*, 2010).

However, some research works had been done in the field of flavour improvement by Maillard reaction of hydrolyses. The Sunflower protein hydrolysates not only had the strong reducing power and DPPH radical scavenging activity, but also had Kokumi taste generated by the high content peptides in the range of 1 to 5 kDa in Maillard reaction. The main processing technology is as follows: Sunflower protein was hydrolysed by Alcalase and Flavourzyme, and the hydrolysates were mixed with the 10% D-xylose and/or 10% L-cysteine. Then the mixtures were heated 2hr at 120℃ in the sealed Pyrex vials to produce the MRPs of Sunflower protein hydrolysates (Eric *et al.*, 2013). Similarly, (Ogasawara *et al.*, 2006) investigated 1000 to 5000 Da peptides from soybean protein hydrolysates heating with xylose at 95℃ for 3.5h and found that the Maillard peptide enhanced the flavour of umami, continuity and tastefulness in consommé soup.

The factors of Maillard reaction that influence flavour formation and, thus, the sensory properties of process flavours, are the type of sugar and amino acid, pH, reaction media, water activity as well as temperature and time. (Taylor & Linforth, 2010). Meat flavour produced by the Maillard reaction was improved with temperature increasing, otherwise it generated the broth-like taste of umami and kokumi. The basic meaty flavour with nutty/roast was attribute to pyrazines, and the umami taste was contributed by Glucose which produced during the low temperature heating processing. The molecular weight of peptide participated in the Maillard reaction below 500Da was benefited to the producing of pyrazines and 2-furfurylpyrrole (Liu *et al.*, 2015).

The MRPs with strong meat and seafood flavour and kokumi was obtained by the Maillard reaction of xylose and Chinese shrimp waste hydrolysates with the controllable processing conditions (Cai *et al.*, 2016). In order to enhance the flavour of chicken hydrolysates, the influence factors of the Maillard reaction between xylose and chicken peptide were discussed (Liu *et al.*, 2015).

It is very useful to increase the additives such as reducing sugars or amino acid during the Maillard reaction of hydrolyses that can enhance the target flavour or mask the initial/creating off-flavours.

Snow crab meat hydrolysates reacted at 72.7℃ for 2 hr with glucose, 5'-IMP, and 5'-GMP produced seafood-like flavour, while reacted at 86.3℃for 3.5 hr with xylose, cysteine, and thiamine at 86.3℃for 3.5 hr generated meat-like process flavour (H.H., J.T., & S.H., 2002). In order to decrease the waste of anchovy sauce residue (ASR), the hydrolysates thermal processing (96℃, 1hr) with fructose (4.0%, w/w), yeast extract (0.5%, w/w) and MSG (0.05%, w/w) additives can mask off-flavor of hydrolysates. And 8 volatiles were correlated with the nutty/baked potato-like odor including 2, 6-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-3, 5-dimethylpyrazine, 2-ethenyl-6-methylpyrazine, 2-isoamyl-6-methylpyrazine, benzothiazole and 2-acetylpyrrole (Cha *et al.*, 2003).

Additives can promote the Maillard reaction not only with the animal protein hydrolysates but also with the plant protein. Adding glucose, ribose, taurine, arginine, alanine, glycine into Seaweed (*Gracilaria fisheri*) protein by-product at the reaction condition of pH: 5.5, 95℃ for 2 hr generated the roasted seafood-like flavouring. Ten volatile flavours were detected and found the dominant compounds were hexanal, hexanoic acid, nonanoic acid, and dihydroactinidiolide (Laohakunjit *et al.*, 2014).

Even though some research works have been done in the field of flavour promotion of protein hydrolyses by Maillard reaction, seldom research in the sediment of hydrolyses. The only reference is (Peinado *et al.*, 2016) used the whole hydrolyses (slurries) to generate seafood flavour.

2.3 Flavour of fish and fish products

Both volatile and involatile components all contribute to the special flavour of various food. Involatile components including free amino acid, peptides, organic acid and nucleic acid derivative attribute to the taste such like umami, sweet, and bitter etc. However, the volatile components play the important role in the aroma or odour. Inosine-5-monophosphate, the umami substance, is attribute to delicious aromas and flavours of very fresh seafood (Damodaran *et al.*, 2007). Delightful aroma or flavour of the very fresh fish attributes to a series of volatile 6-, 8- and 9-carbon carbonyls and alcohols generated by lipoxygenase (Lindsay, 1994). While customers always associate the fish with “commercially fresh” aromas and flavours of secondarily developed stale and fishy favours.

With their special flavours, Sea food products made by traditional processing technologies such as poached, smoked and salted are tempting global costumes with different cultures, religious backgrounds and taste preferences.

Tian etc. analysed the volatile components of five kinds of poached marine fish including Yellow croaker, eel, codfish, pomfret and pan-fried mackerel by headspace solid phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS), and identified five volatile components involving 1-penten-3-ol, 1-octene-3-ol, pentanal, hexanal and 4-heptenal for each species. Compared with the other four species of marine fishes, cod has the unique characteristic volatile flavour components of dimethyl- disulfide, 1-octen-3-one, 2-pentenal, 2-hexenal, 2-octenal, 2-butanone, propanal,2-methyl, ethanone,1-cyclohexyl-,

butanoic acid, 3-methyl ethyl ester and s-methyl, 3-methylbutanethioate (Tian *et al.*, 2015). Among them, 1-octen-3-ol detected in raw cut mushroom has flavours like mushroom and green vegetables which are considered to be the source of the muddy taste in aquatic products. Benzaldehyde which has aromas of bitter almond, cherry and nutty is the specific flavour component of roast peanut and the relative content is highest levels in cod (11.2%). 2-undecanone, with its waxy, fruity, fatty grease and jack fruity flavour, contributes to the flavour of eel and cod. 1-octen-3-one having the strong odours of muddy, mushroom and metallic is the body of the unique flavour compound of cod. Dimethyl disulphide which is only detected in the five species fish has the sharp taste of onion, cabbage and vegetable aroma (Berger, 2007).

Seventy-one components were detected by HS-SPME-GC-MS in Smoked cod with natural smoke, involving ketones and diketones, alcohols, aldehydes, acids, hydrocarbons, esters, ethers, and the derivatives of nitrogenated, phenol, methoxyphenol and dimethoxyphenol. The flavours compounds are not only including ordinary smoked fish flavours such as 2-furanmethanol, benzenemethanol, furancarboxaldehyde, 5-methyl-furancarboxaldehyde, benzeneacetaldehyde and benzaldehyde (detected in cooked mussels (Guen *et al.*, 2000)), but also those from fresh fish (9 to 16 carbon linear aldehydes) (Berger, 2007), from fish degradation processes (propanone), and from fish sauce (1H-pyrrole-2-carboxaldehyde) (Guillén *et al.*, 2006).

Ten phenol compounds, invovling phenol, p-cresol, o-cresol, guaiacol, 4-methyl guaiacol, 4-ethyl guaiacol, syringol, eugenol, 4-propyl guaiacol and isoeugenol, were identified as the main characteristic flavour of smoked herring (Se´rot & Lafficher, 2003). The relationship between the smoking techniques and 10 phenolic compounds flavour were researched, and all the smoking technical parameters investigated (smoking time, smoking temperature, fish fillet temperature, smoke generation, deposition of smoked compounds and exhaust valve opening in the smokehouse or voltage applied in the electrostatic tunnel) impacted the odour characteristics of smoked herring (Serot *et al.*, 2004; Cardinal *et al.*, 2006).

Flavours of four salted-dried fishes, namely, karafuto-shishamo (*Spirinchus lanceolatus*), tigertooth croaker (*Otolithes rube*), little yellow croaker (*Pseudosciaena polyactis*) and hairtail (*Trichiurus haumela*) were identified by HS-SPME-GC-MS. The main volatile components of the salted-dried fishes were aldehydes, alcohols, hydrocarbons which developed the characteristic aromas of green grass-fatty grease and fishy. The specific flavour compounds were 3-methyl butanal, hexanal, (z)-4-heptenal, heptanal, benzaldehyde, octanal, nonanal, 1-penten-3-ol, 3-methyl butanol, 1-octen-3-ol, heptanol and trimethylamine (Li *et al.*, 2012).

2.4 Fish sauce

Fish sauce is one of important condiments in various cuisines in Southeast Asia and the coastal regions of East Asia including China, Cambodia, Philippines, Thailand, Laos, Vietnam, Japan and South Korean. It is an amber-coloured liquid prepared from the fermentation of small marine fish (such as anchovy) with sea salt (Fish Sauce).

Traditional processing method of Chinese fish sauce which is called Yu-lu in Chinese is clear brown liquid extraction of anchovies' fermentation by autolysis normally for several months to one year. The flavour attributes for Yu-lu are meaty, sour, umami, salty, ammonia, cheesy, fecal, fishy, rancid and roasty (Jiang *et al.*, 2007). Jiang etc. analysed the volatile flavour compounds in the four kinds of Yu-lu sampled from one of Yu-lu factories in Fujian by the HS-SPME

combined with GC-MS and found that the major volatile flavour compounds are nitrogen compounds, sulphur compounds and aldehyde components. TMA, sulphur ether compounds and some aldehyde components are the main volatile components and the organic acid is attribute to improve the flavour of Yu-lu and enhance fishy flavour (Jiang & Wang, 2008). The distinctive odour of fish sauce was detected by (Fukami, et al., 2002) is due to four compounds (2-methylpropanal, 2-methylbutanal, 2-ethylpyridine, and dimethyl trisulfide), and these four compounds are contributors to the sweaty aroma too. Among these four compounds, the 2-ethylpyridine and dimethyl trisulfide contribute to the fishy aroma and fecal note.

2.5 Quantitative Descriptive Analysis (QDA)

Sensory evaluation is an important method to assess the quality and quantity of the flavour, taste and the flavour etc. aspects of food and is often used in the food quality control and the new food product development. The methods of scientific sensory evaluation must be carried out under the conditions carefully designed. And before the trained panellists organized, the problem solved must be defined. During the sensory evaluation, a sensory panel of panellists or inspectors is organized to analyse followed by the clear and descriptive guidelines (Rehbein & Oehlenschläger, 2009).

Quantitative Descriptive Analysis (QDA) is used as descriptive sensory analysis for sensory characteristics with qualitative or quantitative results (Pimentel, Madrona, & Prudencio, 2015). It makes sensory evaluation simple to use the attributes. However, the attributes used must be clearly defined, and the words for describing attributes must be cognitively clear too. In ISO 6564 (1985), there is a flavour profile. The panellists need intensive training and a detailed briefing before each group of sensory evaluation. The results of QDA are usually expressed in the form of radar charts.

QDA is practical and widely used to sensory evaluation in fish sauce odour (Fukami *et al.*, 2002; Jiang *et al.*, 2007), cod products related to consumer preferences (Sveinsdóttir, et al., 2009), Arctic charr fillet (Gine´s *et al.*, 2004), durian fruit storage (Voon *et al.*, 2007), apple juice clarified (Pimentel *et al.*, 2015) and consumer preference of dry fermented lamb sausages (Helgesen *et al.*, 1997), etc.

2.6 Response surface methodology (RSM)

Response surface methodology (RSM) is normally applied to explore the relationships between several independent variables (or input) and one or more response (or out-put) variables. It is generally based on the experimental results obtained through a sequence of designed factorial experiments to estimate a first-degree polynomial model and to determine the variables effect on the response variable(s) of interest (Response Surface Methodology).

It has been successfully applied in the optimization of processing parameters. (Harkouss *et al.*, 2014) explored the interaction between temperature, water and salt content on the time course of proteolysis in laboratory-prepared pork meat samples by RSM based on a Doehlert design. To research the impact of the two basic ingredients sugar (10–30%) and coconut milk (15–35%) to responses of textural characteristics (hardness and chewiness) and sensory qualities (colour, firmness, cassava flavour and overall acceptability) of cakes, the formulation for production of a Malaysian traditional baked cassava cake was optimized using RSM based on two-factors central composite (face-centred) design.

RSM was also documented in researching the optimization of MR conditions and flavour formation. In order to improve the emulsifying ability and antioxidant activity, RSM was used to evaluate the effect of heating time, temperature and initial pH on casein–glucose Maillard reaction products (MRPs), and the responses targeted as emulsifying ability and reducing power were taken as response. The processing parameters of MR were optimized based on experiments by Box–Behnken designs (Gu *et al.*, 2009). The effects of initial pH, sonication duration, and ultrasound intensity on the production of Maillard reaction flavour compounds ultrasound assisted in a cysteine-xylose model system were evaluated by RSM based on Central Composite Design (CCD) experimental design (Ong *et al.*, 2015).

3 MATERIALS AND METHODS

3.1 Chemicals

Protamex ™ and Flavourzyme ™ were purchased from Novozymes A/S (Denmark). Optimal working conditions of Protamex ™ (endoprotease declared activity of 1.5 AU/g) are at pH 5.5-7.5 and at 35-60°C. Optimal pH and temperature of exopeptidase of Flavourzyme ™ (endoprotease and exopeptidase declared activity of 500 LAPU/g) are 7.0 and 50 ℃ respectively.

Glucose, xylose, ribose, fructose and lactose monohydrate were all analytical grade (Sigma-Aldrich, U.S.A.).

3.2 Cod cut-offs

Cod (*Gadus morhua*) cut-offs were purchased from the cod fillets company (Samherji hf.). Cod was captured from the North Atlantic Ocean on 3rd January 2016 by Björgulfur trawl and was stored at chilling temperature (-1℃). Cod cut-offs, by-products of fillets, were produced and delivered in heat-insulating boxes containing ice bags to the laboratory of Akureyri University in 15 minutes at 11:30 a.m. on 5th January. Followed by cod cut-offs were stored 12 hours at 4℃in the refrigerator of laboratory.

3.3 Preparation of cod cut-off hydrolysates (CCHs)

Cod cut-offs were minced by meat mincer. The CCHs were prepared by the method of (Slizyte, Rustad, & Storrø, 2005) with corresponding modifications. The cod cut-off mince was preheated at 55℃ for 15mins in the rotary evaporator (30L). The CCHs were prepared by adding 1% Protamex ™ (w/w) for 1 hour, and 1% Flavourzyme ™ (w/w) for next 3 hours hydrolysis at 55℃. During enzymatic hydrolysis, CCHs of 1, 2, 3 and 4 hours were collected for subsequent analysis. After CCHs were cooled to the room temperature, they were frozen at -18℃until further use.

3.4 Development of seafood flavour

Seafood flavour products were prepared by the method reported by (Cai *et al.*, 2016) with modifications. CCHs were unfrozen in water bath and heated at 90℃ for 15mins to inactivate the enzymes. Then they were heated with different 1% sugars (glucose, xylose, ribose, fructose

and lactose) at 90℃ for 0.5, 1, 1.5, 2, 3, 4 hours respectively (Table 3). After reaction, the solution was cooled to the room temperature and then frozen at -18℃ for further analysis.

Thermal degradation products (TDPs) were prepared in the same way as described before without adding sugar as controls.

3.5 Chemical composition

10 g of cod cut-off mince were used to determine water content by the oven-drying method (105℃) (AOAC 2000).

10 g cod cut-off mince which was used to determine ash content was put into the crucible with a lid and heated in the oven (105℃) for 1 hour. Followed by heated on the Bunsen burner in a fume hood until no more smoke is evolved from the sample. Then put the crucible with a lid and prepared sample into the muffle furnace (550℃) together overnight. (AOAC 2000).

0.5 g cod cut-off mince or 1g CCHs was used to determine protein content by Kjeldahl Distilled apparatus. 0.15g CuSO4 was used for the catalyst and 0.1M HCL was for titration.

$$\%\text{Nitrogen}=\frac{(mLTitrant-mLBlank)\times 0.1M\times 1.4007}{sampleweight}$$

$$\%\text{Protein}=\%\text{Nitrogen}\times 6.25$$

10 g cod cut-off mince was used to determine crude fat content by Soxhlet extraction method. The sample was pre-treated by oven-drying to constant weigh. 150 mL (equal volume to the Soxhlet extractor's) re-distilled Hexane was added into the flask as the extraction agent.

Table 3: Experimental groups and Maillard reaction conditions between 1% sugar and FPH.

Samples		Maillard reaction conditions		
		Reaction time (h)	Sugar concentration (%)	Reaction temperature (℃)
Control group (TDP)	C0	0	0	90℃
	C0.5	0.5		
	C1	1		
	C1.5	1.5		
	C2	2		
	C3	3		
	C4	4		
Glucose group	G0	0	1% glucose	
	G0.5	0.5		
	G1	1		
	G1.5	1.5		
	G2	2		
	G3	3		
	G4	4		
Xylose group	X0	0	1% xylose	
	X0.5	0.5		
	X1	1		
	X1.5	1.5		
	X2	2		
	X3	3		
	X4	4		
Ribose group	R0	0	1% ribose	
	R0.5	0.5		
	R1	1		
	R1.5	1.5		
	R2	2		
	R3	3		
	R4	4		
Fructose group	F0	0	1% fructose	
	F0.5	0.5		
	F1	1		
	F1.5	1.5		
	F2	2		
	F3	3		
	F4	4		
Lactose group	L0	0	1% lactose	
	L0.5	0.5		
	L1	1		
	L1.5	1.5		
	L2	2		
	L3	3		
	L4	4		

3.6 The degree of hydrolysis (DH)

The degree of hydrolysis (DH) detected by the method of (Jens, 1982) . The %DH value is the percentage of soluble protein content in trichloroacetic acid (TCA) solution. CCHs were centrifuged at 4700 min/g for 20 mins and the supernatant was collected for DH and amino acid analysis. Equal volume of 10% TCA solution was mixed with 10mL supernatant of CCHs sufficiently and the mixture was placed for 20mins. After the mixture was centrifuged at 4000 g for 15 mins, the nitrogen content in the supernatant and the cod cut-off mince were determined by the Kjeldahl method.

$$\% \text{ DH} = \frac{N_1}{N_0} \times 100$$

Where N_0 represents the nitrogen content in the cod cut-off mince, and N_1 represents the soluble nitrogen content of CCHs in 10% TCA.

3.7 Determination of intermediate products and browning intensity

During the Maillard Reaction, products of intermediate stage are strong absorbed in the ultraviolet. Whereas the products from final stage have the deeper colour than those of the previous two stages (Nursten, 2005). Therefore, the products of intermediate stage and browning degree of MRPs and TDP can be determined at ultraviolet and visible absorption respectively. The method (Maillard *et al.*, 2007) was use for reference with some modifications. The samples were diluted 50 times by distilled water. The absorbance of samples was determined by a UV spectrophotometer (Shimadzu UV-1880, Shimadzu USA manufacturing INC., Canby, or USA) at 294 nm and 420 nm.

3.8 Ultraviolet and visible spectrum scanning of MRPs and TDPs

The supernatant of MRPs and TDPs was diluted by distilled water 50 times. Ultraviolet and visible spectrum scanning of MRPs and TDPs were recorded by a UV spectrophotometer (Shimadzu UV-1880, Shimadzu USA manufacturing INC., Canby, or USA), with the scanned wavelength from 200 to 800 nm.

3.9 Colour measurement

The colour of samples was measured by a BYK Gardner spectro-guide Sphere (6834) Color spectrophotometer (Germany). The colorimeter was calibrated using a standard black of Pantone colour. Ten millilitres of samples were pipetted into a 92×16 mm diameter disposable petri dish. The colour of MRPs and TDPs are expressed by three chromaticity coordinates: L^*(black-white axis), a^* (red-green axis) and b^* (yellow-blue axis). All the data were measured in the same environment (light intensity and background of samples) and repeated five times.

3.10 QDA

The flavour characteristics of samples was assessed by Quantitative Descriptive Analysis (QDA) method of (Fukami *et al.*, 2002) with slight modifications. A panel was formed by eight experienced panellists (4 men and 4 women, age 20–35) who were trained according to international standards (ISO, 1993). The training is including of detecting and recognizing tastes and odours, the use of scales and descriptions. The attributes including salt, sour, bitter, sweet, umami, burnt, fishy and rancid, which were described by (Liu *et al.*, 2015). The panellists were trained to differentiate and reproduce the evaluation assessment results in the replicate samples of Yu-lu. And they can distinguish the intensity of each attribute for a given sample using the different score. The guideline of evaluation was given orally during training sessions according to Yu-lu characteristics. Sensory evaluation was scored along nine scales from -4 to 4, where 0 point was the score of fish sauce product, and -4 is corresponding for a not perceivable intensity while ‘4’ represents for an extreme intensity of an attribute.

The samples were compared with fish sauce product (Blue Dragon™, Thai Pride Brand, Bangkok, Tailand) which salt content is 26.2 g/100g, 12.1 times diluted by water (fish sauce product was diluted to the same salinity with the final product).

3.11 Experimental design for optimization of Maillard reaction parameters

Maillard reaction of CCHs and xylose was optimized by response surface methodology (RSM). The three independent values were xylose concentration (A), reaction temperature (B) and reaction time (C). The effects and interactions of these factors can be evaluated by RSM. Box-Behnken Design was utilized to obtain the optimal combination of variables (Design-Expert 8.0.6). Each independent variable was coded with three levels of -1, 0 and 1. The code levels and actual (Xylose concentration, Temperature and Time) levels of variables were shown in Table 4.

Table 4: Factors and variables of RSM

Levels	Variables		
	A/ Xylose concentration /%	B/ Temperature /℃	C/ Time /h
-1	0.5	70	0.5
0	1.5	80	2
1	2.5	90	3.5

Absorption at 420nm (A_{420}) and 294nm (A_{294}) were chosen as the responses (Y_1 and Y_2) of the Maillard reaction, and QDA of fishy and burnt were chosen as the responses (Y_3 and Y_4) for the flavour formation in the Maillard reaction, respectively. To predict the optimal combination of reaction parameters, the response surfaces (Y_1) was described with the aid of 2FI-order polynomial model according to equation (1) as follows:

$$Y_1=m_0 + m_1A + m_2B + m_3C + m_{12}AB + m_{13}AC + m_{23}BC \quad (1)$$

And the response surfaces Y (Y_2, Y_3, Y_4) was described with the aid of a quadratic-order polynomial model according to equation (2) as follows:

$$Y=n_0 + n_1A + n_2B + n_3C + n_{12}AB + n_{13}AC + n_{23}BC + n_{11}A^2 + n_{22}B^2 + n_{33}C^2 \quad (2)$$

Where Y_1 (Y) is the responses calculated by the model; A, B and C are independent variables, xylose concentration (A), reaction temperature (B) and reaction time (C), respectively; m_0 (n_0) represents an offset term; m_1, m_2 and m_3 (n_1, n_2 and n_3) are the linear effects; m_{11}, m_{22} and m_{33} (n_{11}, n_{22} and n_{33}) are the quadratic and m_{12}, m_{13} and m_{23} (n_{12}, n_{13} and n_{23}) are the cross-product effects of the A, B and C factors on the response. The models were applied to evaluate the effects of each independent variable.

3.12 Production of final product

Millard reaction was proceeded between CCHs and xylose under optimal combination of parameters (Xylose concentration, Temperature and Time) obtained from RSM. After Millard reaction product was centrifuged, the supernatant, that is final product, was obtained.

3.13 PH determination

PH values were measured by pH/ISE Dual Channel Benchtop Meter (Dual Star, Thermo Fisher, America) with a pH electrode (Cole-Parmer, America). PH of cod cut-off mince was measured by the electrode immersing in the mixture of 5g mince and 5mL deionised water at 20 ± 2 °C. For the liquid samples (CCHs and the pilot product), the electrode was immersed in samples

directly to determine pH values. The pH meter was previously calibrated with buffer solutions of pH 10.01 ± 0.01 and 4.01 ± 0.01 at 20 ± 2 °C.

3.14 NaCl content

5ml sample was diluted into 50ml solution by distilled water. 25ml diluted sample pipetted into 150ml breaker was mixed with 25ml 0.5M Nitric Acid ISA solution. The value of electric potential of diluted sample was measured by pH/ISE Dual Channel Benchtop Meter (Dual Star, Thermo Fisher, America) with a Chloride Combination Ion Selective Electrode (Cole-Parmer, America). NaCl standard solutions in a concentration range from 10 to 10,000 ppm/ml (10, 100, 1,000, 5,000 and 10,000 ppm/ml) were used to draw the standard curve. NaCl standard solutions were dealt with the same way as the diluted sample. The standard curve is shown in Figure 2.

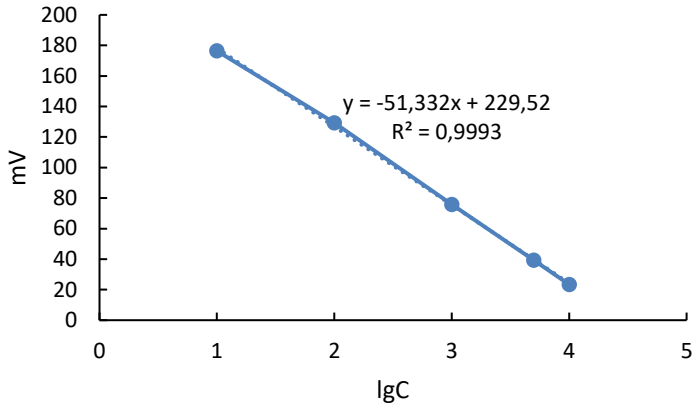

Figure 2: The standard curve of NaCl concentration and mV

$$\%\ \text{NaCl} = \frac{10^{x} \times 58.5 \times 10}{35.5 \times 10{,}000}$$

Where x represents the independent variable according to mV of the sample in the trendline equation.

3.15 Yield of CCHs and final product

The yield_1 of CCHs and the yield_2 of final product were calculated by the equations as follows:

$$\%\text{yield}_1 = \frac{m_1}{m_2} \times 100$$

Where m_1, m_2 represent weigh of CCHs and cut-off mince, respectively.

$$\%\text{yield}_2 = \frac{n_1}{n_2} \times 100$$

Where n_1, n_2 represent weigh of the final product and CCHs, respectively.

3.16 Nitrogen recovery

Nitrogen recovery was used as an indicator to evaluate the soluble nitrogen for the hydrolysis reaction. Nitrogen recovery was determined by the method (Pagán, Ibarz, Falguera, & Benítez,

2013) of with sight modifications. After the hydrolysis reaction, the supernatant was separated from the sediment by centrifuging at 4,700 g/min for 30 min. The nitrogen content was determined by Kjeldahl method (A.O.A.C, 2005). Results were correlated with the ones from the. The NR was calculated by the following formula:

NR (%) = (%) N in supernatant×100/ (%) Total N in cod cut-off mince

3.17 Statistical analysis

All experimental data were carried out in duplicate or triplicates, the mean values and the standard deviation were concluded. One-way analysis of variance (ANOVA) was performed. Duncan's multiple range test was used to compare the significant difference between the different sample groups. The level of significance difference was defined as 5% ($p<0.05$). Statistical analysis was performed with SPSS 19.0 (IBM, Armonk, USA).

4 RESULTS AND DISCUSSION

The raw material used in this study was cut –offs and trimmings from cod fillet processing. And the chemical composition of such raw material was showed in Table 5.

Table 5: Chemical composition of cod cut-offs mince

Chemical compositions	Water (%)	Protein (%)	Ash (%)	Crude Fat (%)
Cod cut-offs mince	80.4±0.41	17.8±2.1	1.44±0.22	0.36±0.15

The results are similar to the previous references except a little bit fluctuation of cod captured from different seasons and different areas. (Shahidi *et al.*, 1991; Liaset & Espe, 2008; Jensen *et al.*, 2013).

4.1 CCHs

The use of proteolytic enzymes has been shown to be an effective way to recover proteins from fish by-products (Bechtel, 2003; Liaset & Espe, 2008; Peinado *et al.*, 2016; Slizyte *et al.*, 2005). Depending on the intended use of the resulting fish protein hydrolysates (FPH), different enzymes and conditions can be chosen, however the $yield_1$ of hydrolysates, degree of hydrolysis (DH) and nitrogen recovery (NY) are three important criteria used to judge the process.

The two enzymes used, effectively dissolved the raw material and only approximately 2% did not hydrolyse after 4 hours' enzyme digestion (Table 6).

Table 6: $Yield_1$ of CCHs

Cod cut-offs mince (kg)	CCHs (kg)	Bones and debris (kg)	Yield (%)
18.7	18.3	0.4	97.8

The heating of the FPH in order to inactivate the enzymes also resulted in denaturation of other proteins and fractionation of the solution into two phases: a clear supernatant phase and a sediment phase (Figure 3).

Figure 3: Sediment and supernatant of CCHs

Figure 4 shows the solids content in the mixture (measured in Brix°) after different reaction times. After one hour the Brix was 18.4 which corresponds to the protein content of the raw material. The Brix value increased to approximately 21° after addition of Flavourzyme®.

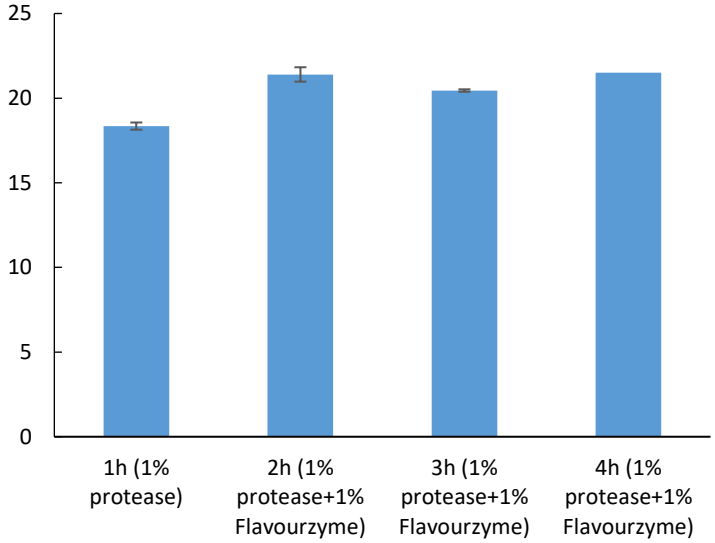

Figure 4: Brix of hydrolysates by different reaction time

Figure 5 shows the distribution of solids in the two different phases. After four hours 67.8% of solids were found in the supernatant. As most of the solids in the cut offs are proteins it can be assumed 75% of the initial protein mass is in the supernatant or approximately 13g/100mL. This was confirmed by nitrogen recovery and protein analysis (Figure 6).

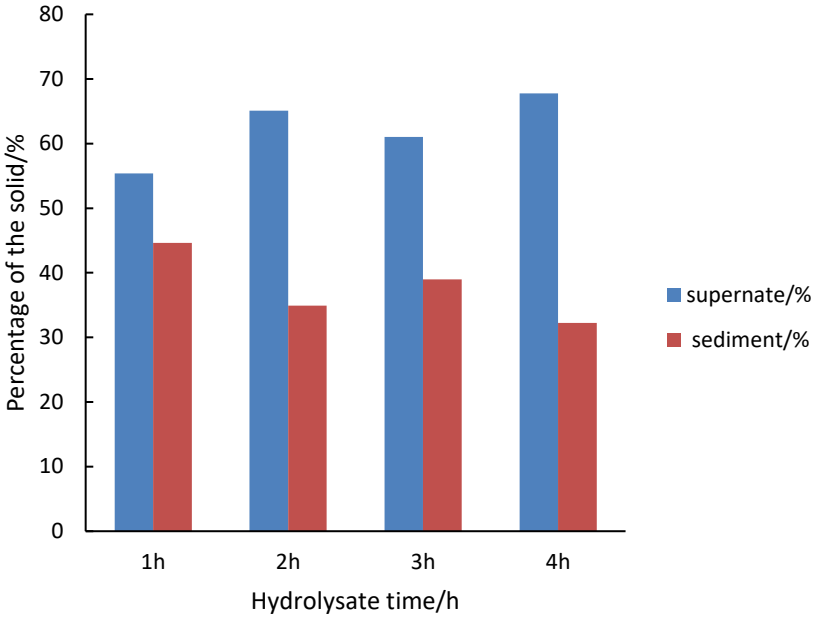

Figure 5: Solid content ratio of supernatant and sediment for different hydrolysis time.

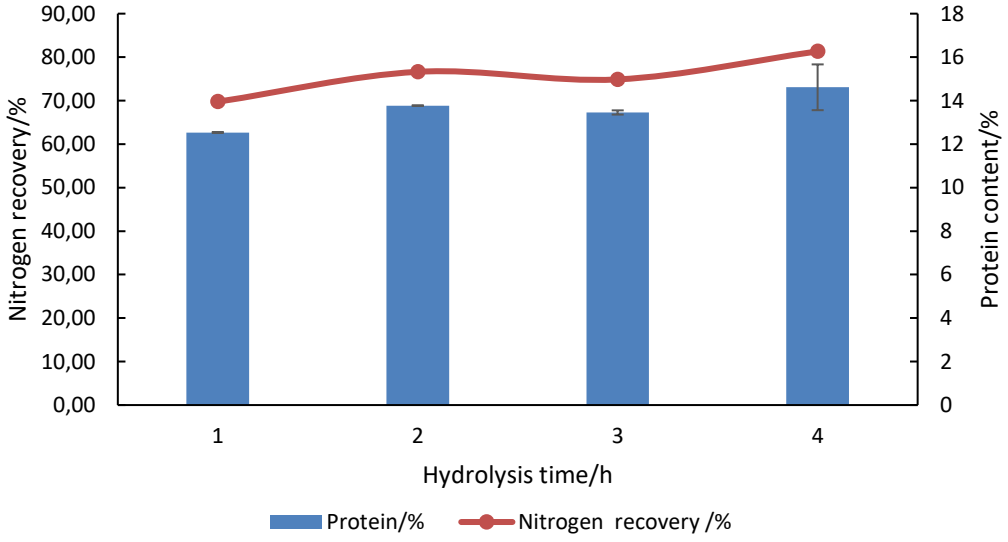

Figure 6: Protein content and nitrogen recovery in the supernatant of different hydrolysis times.

The proteins in the supernatant are partly free amino acids and small peptides resulting from the hydrolysis as well as soluble proteins. To get an estimate on how much of the initial protein has been hydrolysed un-hydrolysed proteins were precipitated using TCA the remaining N-content (non-protein N and peptide nitrogen) used to estimate the degree of hydrolysis (DH). Figure 7 shows the effect of time on the DH of cod-cut offs using Protamix® and Flavorzime® mixtures. The figure shows that hydrolysis times above 2 hours did not result in higher DH. The values obtained are comparable with the findings of Silva and Pagán (Silva, Ribeiro, Silva, Cahú, & Bezerra, 2014; Pagán, Ibarz, Falguera, & Benítez, 2013).

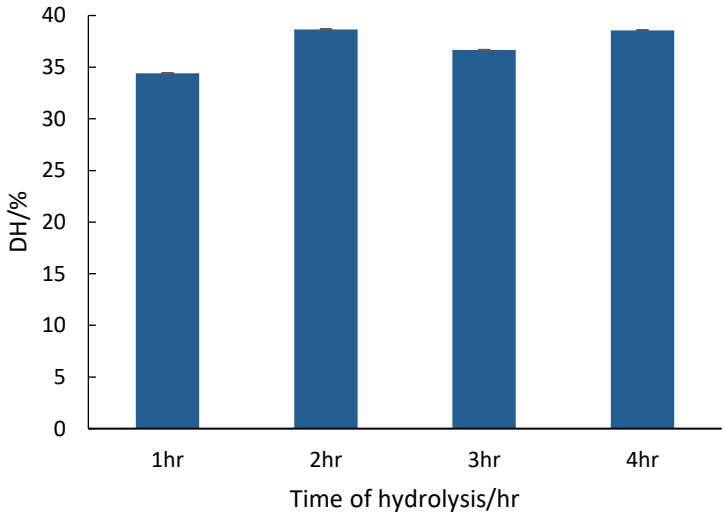

Figure 7: Effect of time on the degree of hydrolysis (DH) of cod-cut offs using combination of Protamix / Flavorzyme (1% / 1%) mixture at 90°C

4.2 Effects of different sugars on browning, colour and flavour formation in FPH

4.2.1 UV absorption (294nm)

The products from the intermediate stage of MR are series of uncoloured compounds which have the absorbance at 294 nm. Therefore, it is usually used as an indicator as the formation of the MR precursor (Nursten, 2005). However, the absorbance at 420 nm is often used as an indication for the intensity of brown colour formation in the advanced stage of MR. The effect of reaction time on the intermediate stage of MR from different sugars is shown in Figure 8.

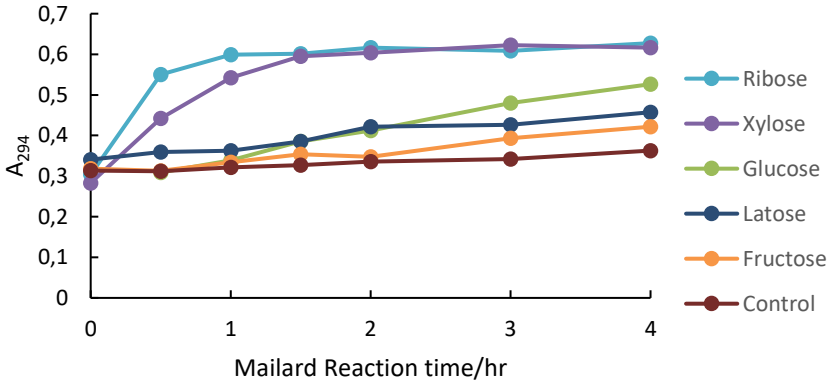

Figure 8: A294 (in 1:50 dilution) of CCHs heating (90°C) with different sugars

It is clear that ribose and lactose react much faster than glucose, lactose and fructose. Fructose, glucose and lactose reacted slightly faster than the control group (heated without added sugars). Extension of reaction time could form intermediate products in fructose and lactose mixtures. Absorbance at 294nm increased markedly in the glucose mixture after 1hour reaction. Most significant increase of absorbance at 294nm was in ribose and xylose mixtures. The absorbance at 294nm increased rapidly from start until 1.5h, were after little changes were observed

indicating equilibrium in the development of intermediate products. The production from ribose was more rapid than that from xylose in the first 1 hour. No changes occurred in the control group (TD).

4.2.2 UV absorption (420nm)

The reaction time effecting on the final stage of MR from different sugars was shown in Figure 9. In fructose and lactose group, with the reaction time extension, the absorbance at 420nm were almost no change as control group (TD). It suggested that MR from Fructose and Lactose did not reach the final stage even after 4 hours' reaction and the colour of the samples in these group were no changes as the control group. There was slightly change of absorbance at 420nm in glucose group. The final stage of MR from glucose began from 1.5 hours. However, MR from ribose and xylose were more active than other groups especially in the initial 2 hours which the intensity of brown colour development were increasing rapidly (Damodaran *et al.*, 2007).

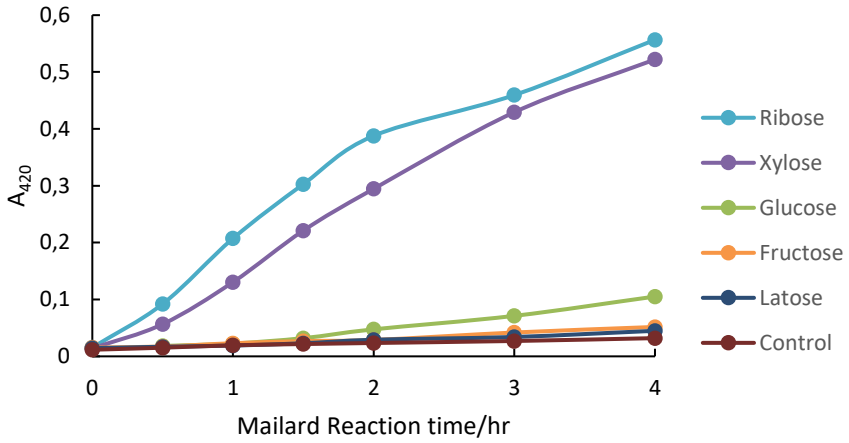

Figure 9: Absorption at 420nm (in 1:50 dilution) of CCHs heating (90°C) with different sugars.

4.2.3 Ultraviolet-Visible (UV-Vis) Scan

UV-Vis scan is commonly used to study the character of MRPs. Spectra of MRPs different groups and TD scanned by UV-Vis spectrophotometer from 200 to 800 nm is shown in Figure 10. The UV-Vis spectra from the different sugar mixtures had very similar shapes, that is, there was a peak at ~290nm and then the absorbance decreased at higher wavelengths. With increased reaction time, the maximum absorbance of MRs was increased in each sugar mixture, especially in the ribose and xylose mixtures with maximum absorbance from 0.310 to 2.355 and 0.322 and 2.569 respectively. In addition, during the first 0.5h to 2h of reaction time, the maximum absorbance of MR increases more rapidly than the last 2h of the reaction. It can also be noted that there was a smaller peek at around 420-450nm (indication of advanced browning) in the xylose and ribose mixtures. The result is consistent with the study that the rate of the Maillard reaction is faster for pentoses than hexoses (Nursten, 2005).

The wavelength and maximum absorbance of different sugars for different reaction time is shown in Table 7. With extended reaction times, the maximum absorbance wavelength was more stable at 296nm in control, lactose and fructose group; the maximum absorbance wavelength was slightly redshifted from 296nm to 300nm in glucose group; while in the ribose and xylose group, the maximum absorbance wavelength obviously redshifted from 298nm to 310nm and 296nm to 312nm, respectively. Similarly, it was reported that when the Maillard reaction processes to the final stage, the MRPs would to be redshifted (Nursten, 2005). Also, the result is consistent with Maillard reaction from xylose and Chinese shrimp waste hydrolysates (Cai, *et al.*, 2016).

Colour is one of the most important criteria for food quality and very important to potential customers. During food processing (e.g. during heat treatment), the colour of the food changes often as a result of the Maillard reaction when coloured compounds generated. There is a positive correlation between the increase in the colour of food product and the extension of Maillard reacting time. Colour changes in heated mixtures of CCHs and different sugars is shown in Figure 11. From the figure (Figure 11a), it can be seen that the L values (black-white component, luminosity) of all samples decreased, albeit only slightly in the control group. This indicates that the colour of all samples became darker with increasing reaction time, due to formation of brown compounds in the Maillard reaction.

Table 7: The wavelength and maximum absorbance of different sugars for different reaction time

Reaction Time/h	Control (TD) Wavelength/ nm	Max Abs	Lactose Wavelength/n m	Max Abs	Fructose Wavelength/n m	Max Abs	Glucose Wavelength/n m	Max Abs	Xylose Wavelength/n m	Max Abs	Ribose Wavelength/ nm	Max Abs
0	290	0.313	290	0.363	296	0.333			290	0.310	296	0.322
0.5	296	0.322	290	0.363	296	0.333	290	0.327	298	0.559	306	0.948
1	296	0.347	294	0.362	296	0.367	296	0.364	306	0.885	308	1.641
1.5	296	0.355	294	0.385	296	0.395	296	0.447	308	1.289	308	2.048
2	296	0.366	294	0.422	296	0.392	296	0.494	308	1.620	308	2.325
3	296	0.383	296	0.428	296	0.469	298	0.658	308	2.076	308	2.393
4	296	0.413	296	0.478	296	0.514	300	0.797	310	2.355	312	2.569

This paper should be cited as:
Min, Q. 2018. *Savoury flavour formulations from maillard reaction of hydrolysated cod by-products and sugars.* United Nations University Fisheries Training Programme, Iceland [final project].
http://www.unuftp.is/static/fellows/document/min15prf.pdf

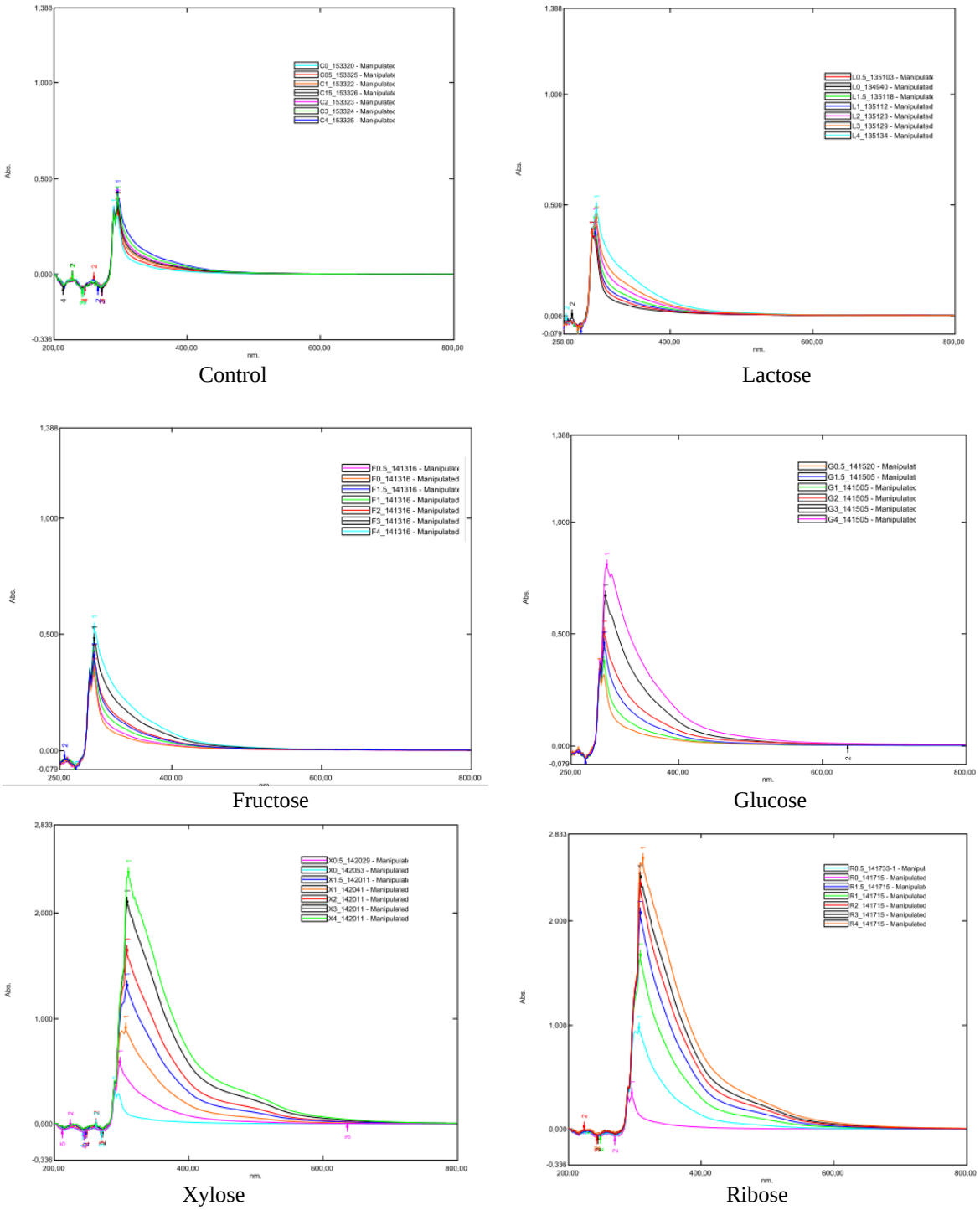

Figure 10: UV-Vis spectra of MRPs formed by heating (80°C) mixtures of CCHs and from different sugars for up to four hours.

4.2.4 4.2.3 Colour of TDPs and MRPs

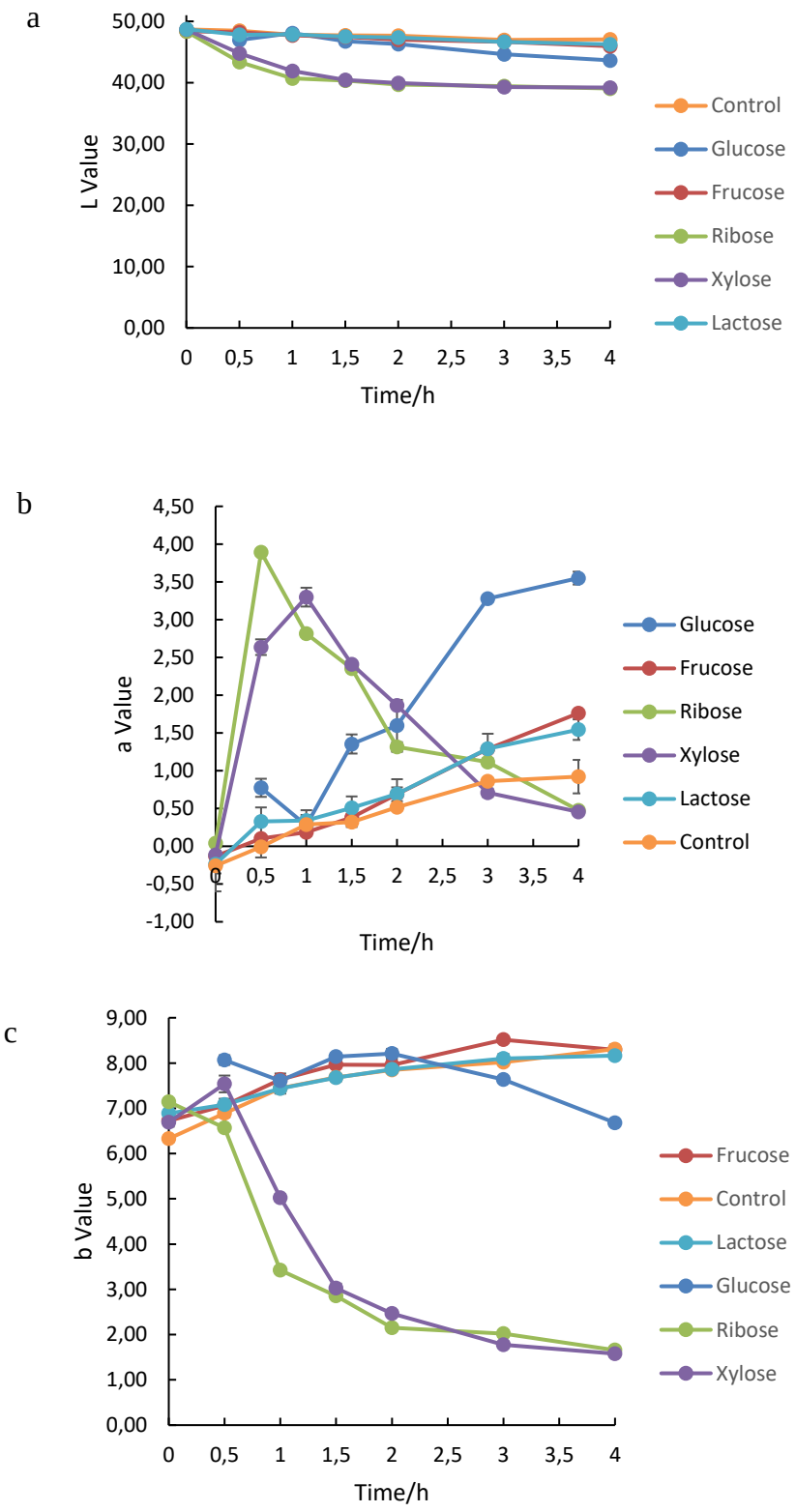

Figure 11: Colour formation of TDPs and MRPs with different sugars for different reaction time (a) Different sugars effect on the L value of MRPs and TD; (b) Different sugars effect on the a value of MRPs and TD; (c) Different sugars effect on the b value of MRPs and TD.

The L values of xylose and ribose mixtures decreased rapidly, that is, the colour of xylose and ribose group were increased rapidly especially in the first hour of the reaction. The result is accordance with the previous research that Maillard reaction is faster in pentose than hexose mixtures.

The a values (green to magenta/red component) of glucose, fructose and lactose mixtures increased and slightly more than in the control group, even though their trend is similar. This indicates that the colour is getting redder with increased reaction time. However, the a values of ribose and xylose mixtures increased significantly to reach the peak after 0.5 to one hour of heating, then the a-value decreased rapidly for the next 2.5 hours and 2 hours, respectively. The b values (blue to yellow component) of fructose, lactose and control group increased slowly. The colour of the three groups changed thus to a slightly more yellow character.

Whereas after 0.5 hour of heating to the peak, the b values of xylose and ribose group were decreased rapidly. The colour of the two group reflected less yellow properties. That is due to the intermediates or final products during the intermediate and third stage of the Maillard reaction.

The control group (TDPs) showed only slight changes in L, b and a values and remained greyish throughout the heating period.

4.2.5 Flavour formation

The flavour formation in the different FPH and sugar mixtures were evaluated in two different steps; first by a flavour expert and then by a semi- trained test panel.

The evaluation by the expert showed that:

- Heating of FPH with different sugars resulted in unique reaction flavours albeit very different between sugars.
- Comparison within sugar mixtures showed enhanced flavour formation from time zero throughout the reaction time (four hours), also the fish flavour of the control group samples increased with the heating time and the flavour of FPH heated for 1.5h was richer than that of non-heated sample (0h)
- Compared with the other mixtures, xylose gave more “exciting” flavour, however its dark colour is a drawback.
- The xylose mixture had a “fish sauce flavour character” but also a sweet tone
- The lactose mixtures had better flavour than the others sugar mixtures and a distinct fish flavour (i.e. the lactose enhanced the original fish flavour) however the flavour vas highly dependent on reaction time.
- Fructose mixtures were bitterer than the other sugar mixtures however here reaction time had little influence.
- Ribose (0h) had the similar flavour as control (0h); both xylose (0h) and lactose (0h) had better flavour than control 0 and the flavour of fructose 0 was weaker than the other samples. After 4 hours heating the ribose mixture had “confused” flavour and was less sweet than xylose (4h).
- Glucose (1.5h) also had a rich flavour, more than lactose (1.5h), however the lactose mixture (1.5h) had “better” flavour.

The overall result of the expert's evaluation was that lactose enhanced the fish flavour and the xylose gave a fish-sauce like flavour.

The panel was asked to evaluate those sugar mixtures (xylose and lactose) heated for one and for four hours. The results are shown in Figure 12 below.

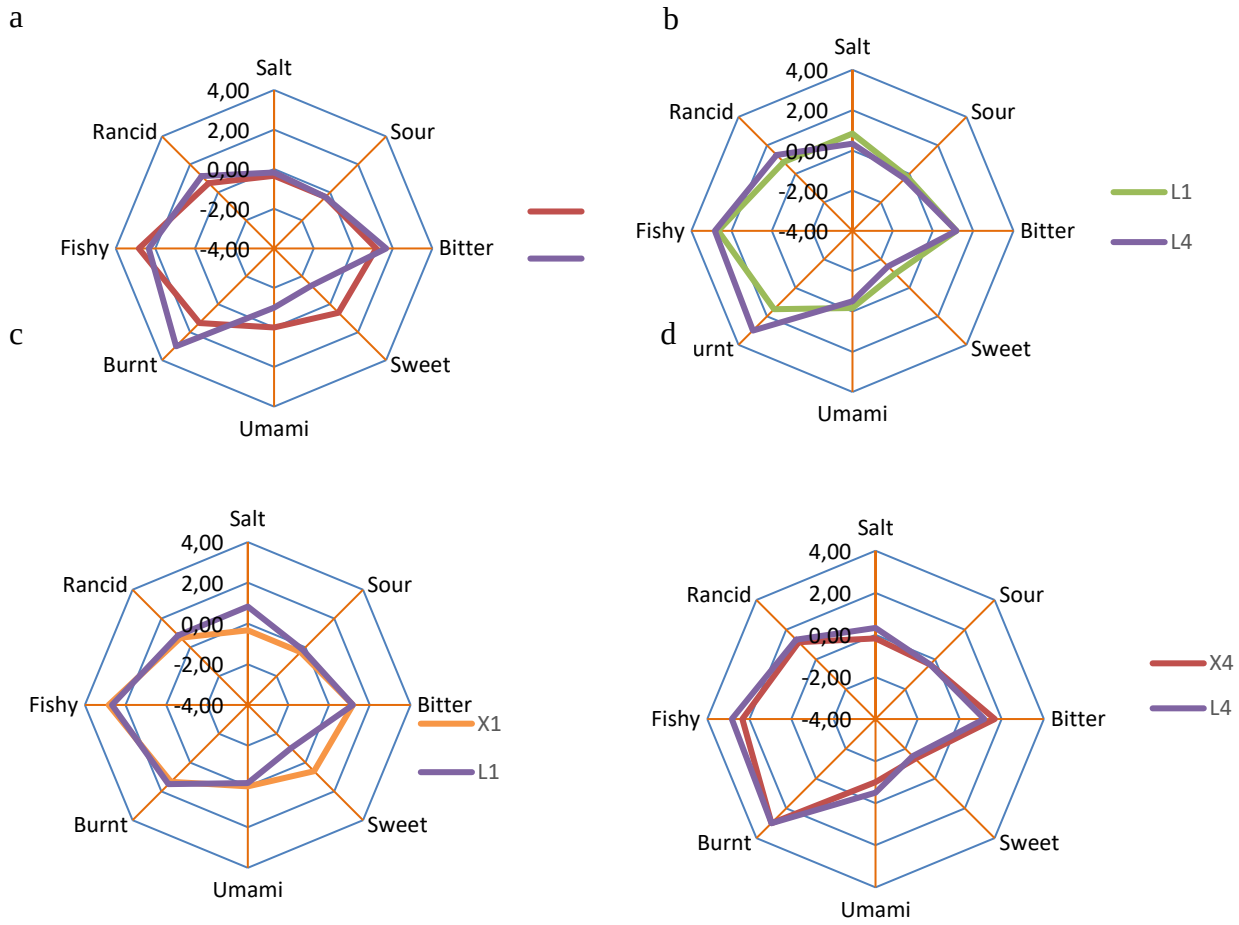

Figure 12: Comparative analysis of the sensory property between Maillard reaction products from different sugars or for different reaction times (a) Comparison between Maillard reaction products from 1% of xylose heating at 90℃ with FPH for 1 hour and 4 hours; (b) comparison between Maillard reaction products from 1% of lactose heating at 90℃ with FPH for 1 hour and 4 hours; (c) comparison between Maillard reaction products from 1% of xylose and 1% of lactose heating at 90℃ with FPH for 1 hour; (d) comparison between Maillard reaction products from 1% of xylose and 1% of lactose heating at 90℃ with FPH for 4 hours.

The comparison between Maillard reaction products of different sugars or different reaction times were showed in Figure 12.

The FPH-sugar mixtures were compared with fish-sauce (1:10 diluted to equalize salt content). The figure shows that the xylose increased burnt, fishy, rancid and bitter taste however salt, sour and sweet taste notes were similar to the reference. The reaction time increased burnt, rancid and bitter but decreased fishy and sweet notes (Figure 12 (a)).

The lactose gave similar results exempt that the fishy note is similar after one and four-hour reaction (Figure12 (b)).

After the first 1-hour Maillard reaction, xylose gave the sweet note, while lactose increases the salty taste (Figure 12 (c)). The most likely explanation might be sugar has different relative sweetness at the same concentration. The relative sweetness of xylose and lactose is 0.7 and 0.2, respectively (Damodaran *et al.*, 2007). With reaction time increasing, Caramelization reaction followed by the Maillard reaction, burnt and bitter were increasing and became the keynote flavour of samples (Figure 12 (d)).

4.3 Reaction kinetics in FPH and xylose mixtures

In order to study the effect of time, temperature and sugar concentration on the Maillard reaction (Abs294 and Abs420) flavour formation (fishy and burnt) an experiment (17 runs) was designed by Box–Behnken response Surface Method and the resulting 17 runs were carried out in a random order. The results and the responses of the experiments are shown in Table 8, while characteristics of models for A_{294} and A_{420} are given in Tables 9 and 10, respectively. The ANOVA confirms statistical adequacy of the models for the responses since the values of prob > F are less than 0.05 that indicate the models are statistically significant at the 95% confidence level. Meanwhile in the Quadratic model for A_{294}, A, B, C, AB, BC, A^2, B^2 and C^2 are significant terms (Table 9). And in the 2FI model for A_{420}, A, B, C, AB, AC and BC are significant terms (Table 10). In addition, the models express high determination fitting coefficients (R^2) and low variation coefficients (CV) as follows: R^2=0.9905 and CV=3.05 for A_{294}; R^2=0.9641 and CV=26.11 for A_{294}. These results of ANOVA suggest that the model which obtained from the experiments designed is reliable and accurate. The fitting model equations are as follows:

$$A_{294} = -2.43291+0.34486A+0.049414\ B+0.35297C-(1.97500E-003)\ AB+(3.05000E-003)\ AC - (2.19500E-003)\ BC-0.048575\ A^2-(2.09750E-004)\ B^2-0.028844\ C^2 \quad (3)$$

$$A_{420}= +2.07200 -1.65141\ A-0.025639\ B-0.88263\ C+0.020445\ AB+0.11107\ AC +0.010893\ BC \quad (4)$$

Table 8: Scheme design and experiment results of RSM

Run	A Xyclose concentration/%	B Reaction temperature/℃	C Reaction time/h	A_{294}	A_{420}	Fishy	Burnt
1	0.50	80.00	0.50	0.334	0.0217	1.43	0.86
2	1.50	80.00	2.00	0.5973	0.2852	1.29	2.57
3	1.50	80.00	2.00	0.5973	0.2852	1.29	2.57
4	1.50	80.00	2.00	0.5973	0.2852	1.29	2.57
5	1.50	70.00	0.50	0.3117	0.0198	1.57	0
6	1.50	90.00	0.50	0.5378	0.1181	1.71	1.86
7	0.50	70.00	2.00	0.365	0.0303	2.14	1.43
8	1.50	80.00	2.00	0.5973	0.2852	1.29	2.57
9	2.50	80.00	3.50	0.6428	0.8579	0.71	3.43
10	2.50	80.00	0.50	0.4076	0.0418	0.57	1.29
11	2.50	90.00	2.00	0.651	1.1498	0.86	3.57
12	1.50	90.00	3.50	0.6453	0.8946		
13	2.50	70.00	2.00	0.5107	0.0934	2.43	1.71
14	1.50	80.00	2.00	0.5973	0.2852	1.29	2.57
15	1.50	70.00	3.50	0.5509	0.1427	2.14	1.86
16	0.50	90.00	2.00	0.5843	0.2689	1.43	3.43
17	0.50	80.00	3.50	0.5509	0.1714	1.14	2.43

The absorbance 294 nm (A_{294}) is usually used as an indicator of the formation of uncoloured compounds supposed to the precursor of MR in the intermedia stage, and the absorbance at 420 nm (A_{294}) is often used as an indication of the intensity of brown colour produced in the final stage of MR (Kim & Lee, 2009). Therefore, the Equation (3) and (4) describe the relationships between the parameters (xylose concentration, reaction temperature and reaction time) and the MR between xylose and FPH in some ways.

4.3.1 Response of A_{294}

Table 9: Variance analysis of A_{294}

c	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.19	9	0.022	81.23	< 0.0001	significant
A-xylose concentration	*0.018*	*1*	*0.018*	*67.37*	*< 0.0001*	
B-reaction temperature	*0.058*	*1*	*0.058*	*218.21*	*< 0.0001*	
C-reaction time	*0.080*	*1*	*0.080*	*301.03*	*< 0.0001*	
AB	*1.560E-003*	*1*	*1.560E-003*	*5.89*	*0.0456*	
AC	*8.372E-005*	*1*	*8.372E-005*	*0.32*	*0.5916*	
BC	*4.336E-003*	*1*	*4.336E-003*	*16.37*	*0.0049*	
A^2	*9.935E-003*	*1*	*9.935E-003*	*37.50*	*0.0005*	
B^2	*1.852E-003*	*1*	*1.852E-003*	*6.99*	*0.0332*	
C^2	*0.018*	*1*	*0.018*	*66.93*	*< 0.0001*	
Residual	1.855E-003	7	2.650E-004			
Lack of Fit	*1.855E-003*	*3*	*6.182E-004*			
Pure Error	*0.000*	*4*	*0.000*			
Cor Total	0.20	16				
Std. Dev.	0.016	R-Squared			0.9905	
Mean	0.53	Adj R-Squared			0.9783	
C.V. %	3.05	Pred R-Squared			0.8483	
PRESS	0.030	Adeq Precision			29.615	

Table 9 shows that A_{294} is positively related to the linear effect of xylose concentration, reaction temperature and reaction time. The interaction effects of xylose concentration and reaction temperature, the interaction effects of reaction temperature and reaction time, and the quadratic terms of xylose concentration, reaction temperature and reaction time have a negative effect (equation (3)), and these terms significant effects on A_{294}.

Three-dimensional and contour plot figures of interactive effects of the independent parameters on A_{294} are showed in the Figure 13 and Figure 14. It is easy to see the independent parameters (xylose concentration, reaction temperature and reaction time) effects on the absorbance at 294nm (A_{294}), that is, on the generation of uncoloured compounds in the intermedia stage of MR.

From Figure 13(a), it shows that the interactive effects of xylose concentration and reaction temperature on the intermedia stage of MR are curved. In the same way, From Figure 12(b) and(c), the interactive effects of xylose concentration and reaction time and the interactive effects of reaction temperature and reaction time on the intermedia stage of MR. are curved too.

From Figure 14(a), it very clear that the density of contour lines along the axis of xylose concentration is higher than those along the axis of reaction temperature. That suggests the effect of xylose concentration is bigger than that of reaction temperature on the intermedia stage of MR. Similar, from the Figure 14(b) and (c), conclusions can be drawn that the effect of reaction time is bigger than that of xylose concentration, and the effect of reaction time is bigger than that of reaction temperature.

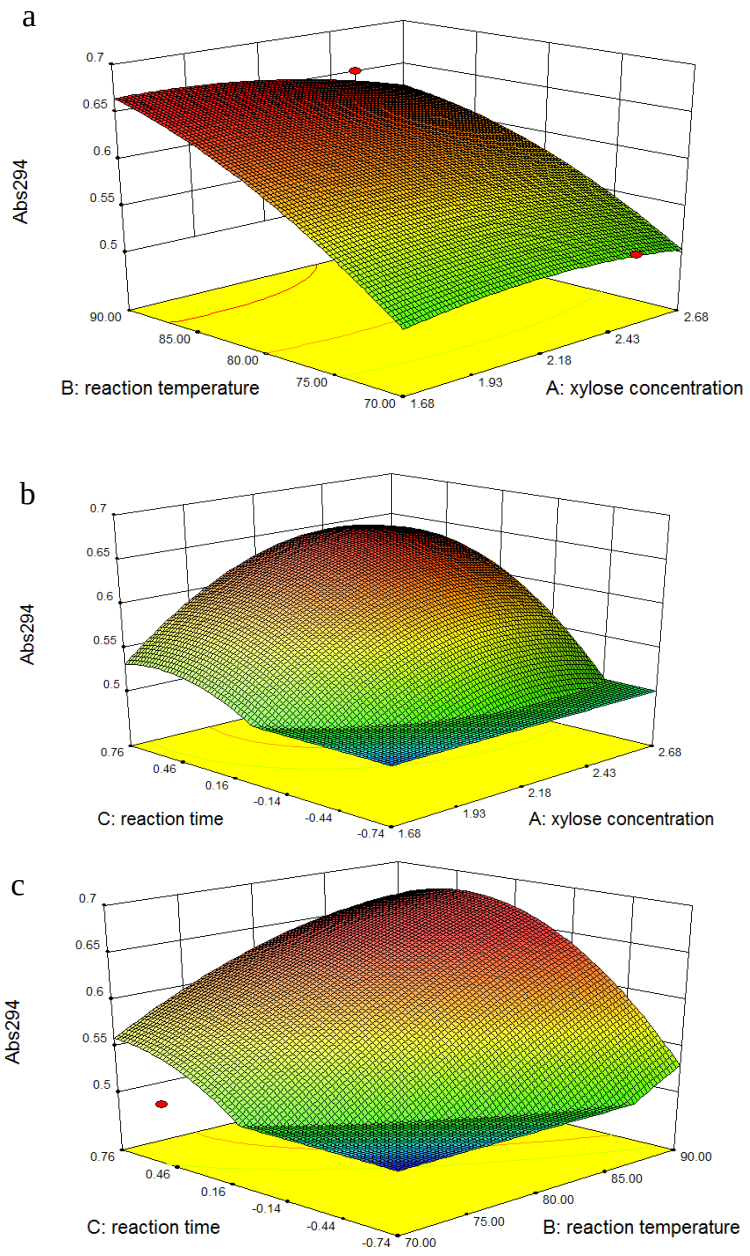

Figure 13:Three-dimensional figures of interactive effects of xylose concentration, reaction temperature and reaction time on Abs294. (a) Interactive effects of xylose concentration and reaction temperature on Abs294; (b) interactive effects of xylose concentration and reaction time on Abs294; (c) interactive effects of reaction temperature and reaction time on Abs294.

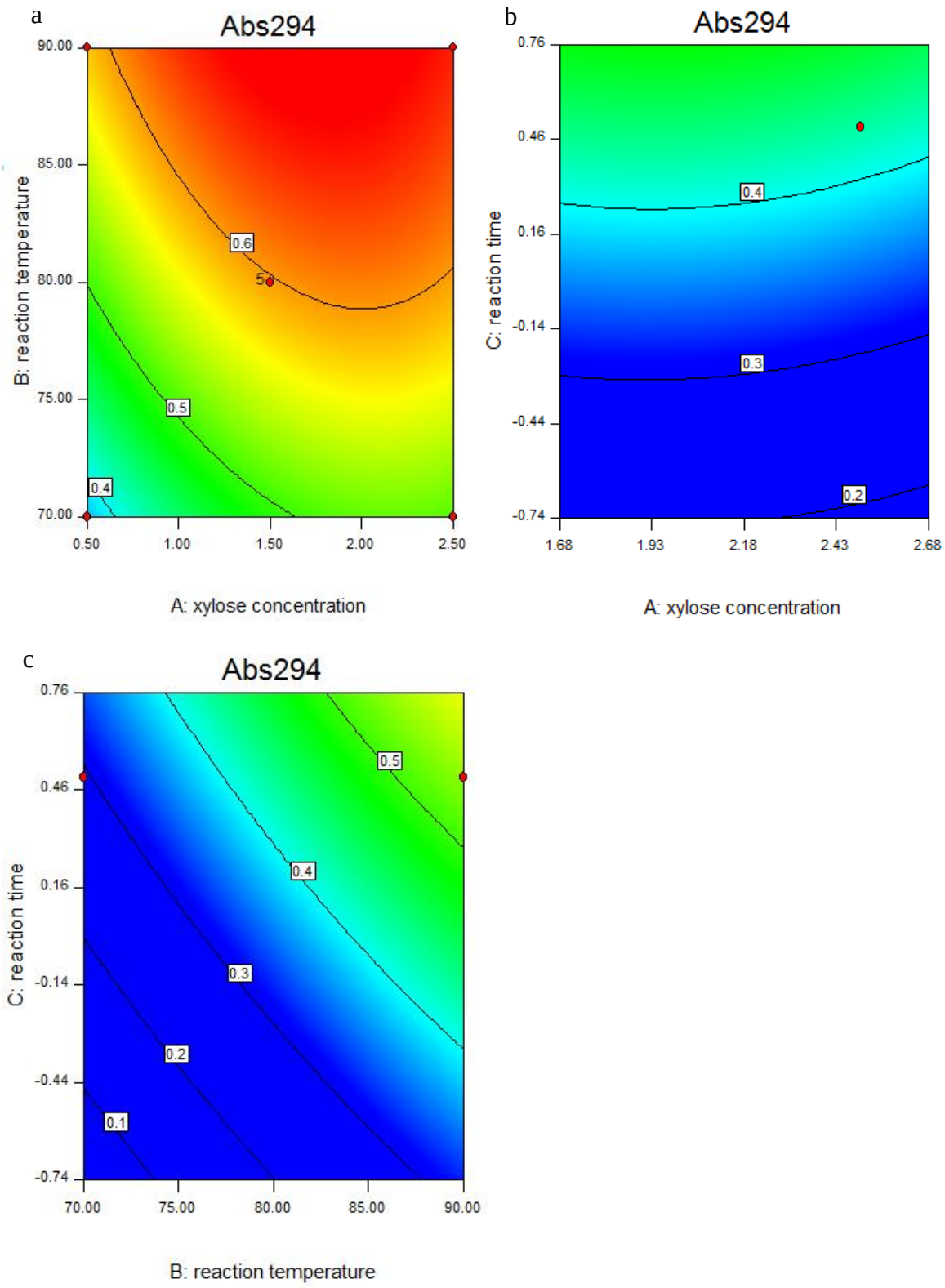

Figure 14: Contour plot figures of interactive effects of xylose concentration, reaction temperature and reaction time on Abs294 (a) Interactive effects of xylose concentration and reaction temperature on Abs294; (b) interactive effects of xylose concentration and reaction time on Abs294; (c) interactive effects of reaction temperature and reaction time on Abs294.

4.3.2 Response of A_{420}

Table 10: Variance analysis of A420

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	*1.74*	*6*	*0.29*	*44.74*	*< 0.0001*	significant
A-xylose concentration	*0.34*	*1*	*0.34*	*52.67*	*< 0.0001*	
B-reaction temperature	*0.58*	*1*	*0.58*	*88.96*	*< 0.0001*	
C-reaction time	*0.43*	*1*	*0.43*	*67.25*	*< 0.0001*	
AB	*0.17*	*1*	*0.17*	*25.86*	*0.0005*	
AC	*0.11*	*1*	*0.11*	*17.17*	*0.0020*	
BC	*0.11*	*1*	*0.11*	*16.52*	*0.0023*	
Residual	0.065	10	6.466E-003			
Lack of Fit	*0.065*	*6*	*0.011*			
Pure Error	*0.000*	*4*	*0.000*			
Cor Total	1.80	16				
Std. Dev.	0.080	R-Squared			0.9641	
Mean	0.31	Adj R-Squared			0.9425	
C.V. %	26.11	Pred R-Squared			0.8213	
PRESS	0.32	Adeq Precision			19.706	

From Table 10, it suggests that A_{420} is negatively related to the linear effect of xylose concentration, reaction temperature and reaction time, however positively related to the interaction effects of xylose concentration and reaction temperature, the interaction effects of reaction temperature and reaction time, and the interaction effects of xylose concentration and reaction time (equation (4)), and these terms significant effects on A_{420}.

Three-dimensional and contour plot figures of interactive effects of the independent parameters on A_{420} are showed in the Figure15 and Figure 16. It is easy to see the independent parameters (xylose concentration, reaction temperature and reaction time) effects on the absorbance at 420nm (A_{420}), that is, on the intensity of brown colour developed from the final stage of MR.

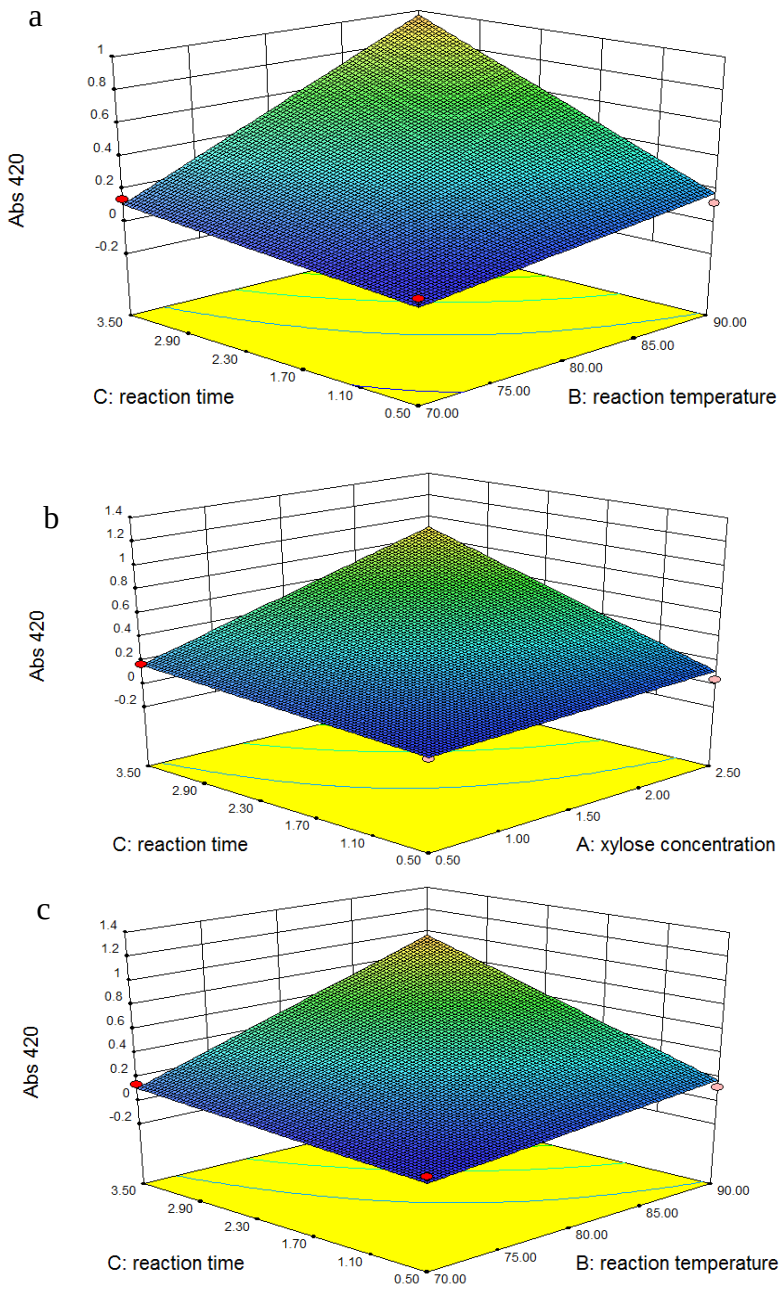

Figure 15: Three-dimensional figures of interactive effects of xylose concentration, reaction temperature and reaction time on Abs420. (a) Interactive effects of xylose concentration and reaction temperature on Abs420; (b) interactive effects of xylose concentration and reaction time on Abs420; (c) interactive effects of reaction temperature and reaction time on Abs420.

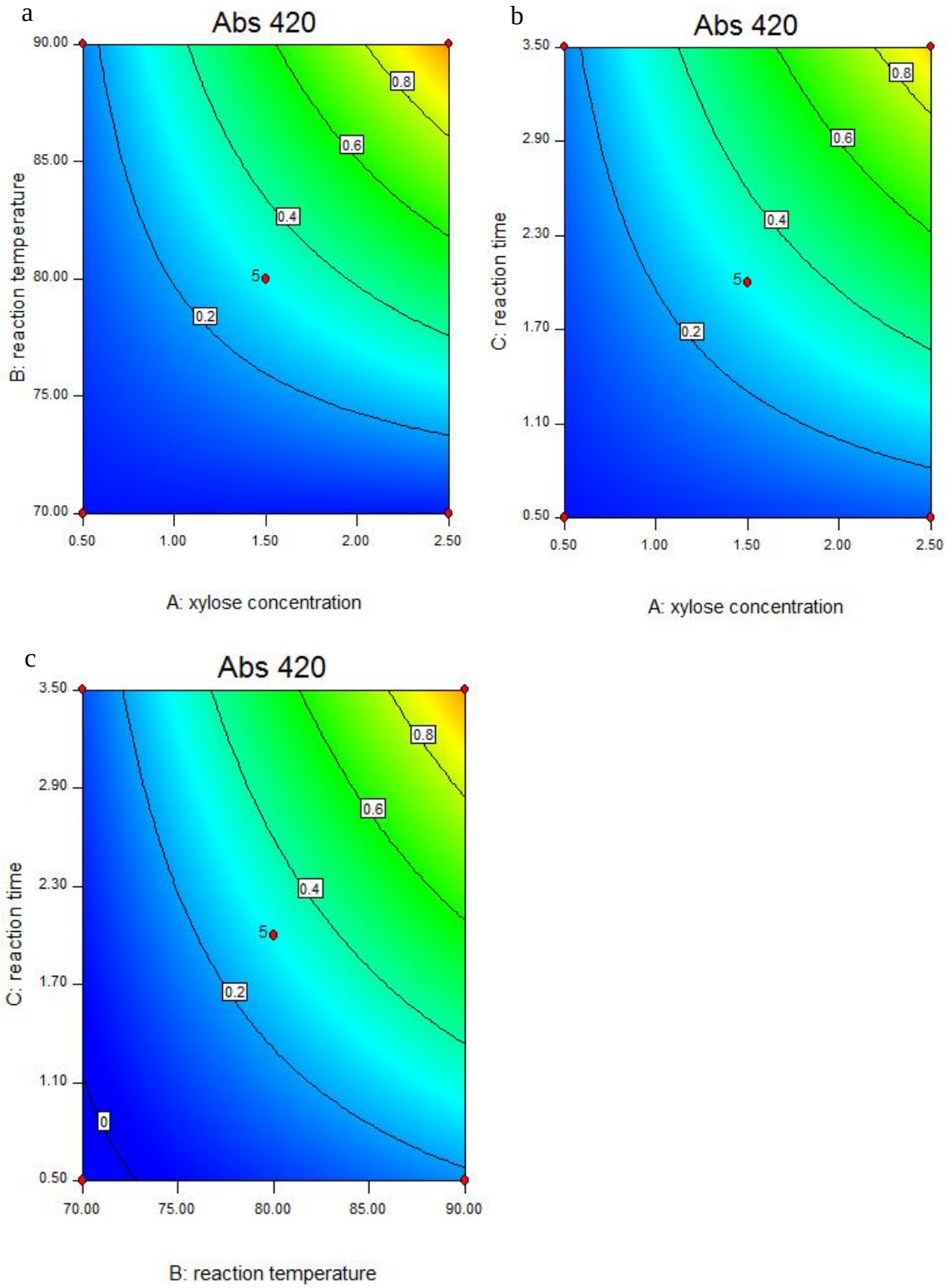

Figure 16: Contour plot figures of interactive effects of xylose concentration, reaction temperature and reaction time on Abs420.n (a) Interactive effects of xylose concentration and reaction temperature on Abs420; (b) interactive effects of xylose concentration and reaction time on Abs420; (c) interactive effects of reaction temperature and reaction time on Abs420.

From Figure 15(a), it shows that the interactive effects of xylose concentration and reaction temperature on the final stage of MR. are nonlinear. In the same way, From Figure 15(b) and(c), the interactive effects of xylose concentration and reaction time and the interactive effects of reaction temperature and reaction time on the final stage of MR. are nonlinear too.

From Figure 16(a), it very clear that the density of contour lines along the axis of xylose concentration is higher than those along the axis of reaction temperature. That suggests the effect of xylose concentration is bigger than that of reaction temperature on the intermedia stage of MR. Similar, from the Figure 16(b) and (c), conclusions can be drawn that the effect of xylose concentration is bigger than that of reaction time, and the effect of reaction time is bigger than that of reaction temperature.

According to the expert opinions, sample of lactose 2h has the best flavour and taste than all of the samples. A_{420} of lactose 2h is 0.2945. Therefore, the response of A_{420} is targeted at 0.2945 for the optimization of RSM. 6 solutions of optimization were found. In consideration of practical production, the shortest production will be welcomed by the factory. Therefore, the optimum conditions for MR from xylose and FPH are as follows, xylose concentration, reaction temperature and reaction time were 1.5%, 90℃ and 0.93h.

4.3.3 Response of fishy flavour formation

The characteristics of models for flavour formation (fishy and burnt) are given in Tables 11 and 12, respectively. The ANOVA confirms statistical adequacy of the models for the responses since the values of prob > F are less than 0.05 that indicate the models are statistically significant at the 95% confidence level. Meanwhile in the Quadratic model for flavour formation (fishy and burnt), A, B, C, AB, BC, A^2, B^2 and C^2 are significant terms (Table11 and Table 12). In addition, the models express high determination fitting coefficients (R^2) and low variation coefficients (CV) as follows: R^2=0.9409 and CV=13.82 for fishy; R^2=0.9909 and CV=6.75 for burnt. These results of ANOVA suggest that the model which obtained from the experiments designed is reliable and accurate. The fitting model equations are as follows:

Fishy = +26.51737+1.46479 A-0.67765 B+2.91347 C-0.021500 A B+0.071667 A C-0.032083 B C-0.028125A^2+4.53125E-003 B^2-0.13306 C^2 (5)

Burnt = -12.78005+0.18438 A+0.22765 B+1.48542 C-(3.50000E-003) A B+0.071667 A C-0.032083 B C-0.028125A^2+4.53125E-003 B^2-0.13306 C^2 (6)

Therefore, the Equation (5) and (6) describe the relationships between the parameters (xylose concentration, reaction temperature and reaction time) and the flavour formation (fishy and burnt) of MR between xylose and FPH.

Table 11 shows that the formation of fishy flavour is positively related to the linear effect of xylose concentration and quadratic terms of reaction temperature. The linear effect of reaction temperature, the interaction effects of reaction temperature and reaction time, and the quadratic terms of reaction time have a negative effect (equation (5)), and these terms significant effects on the formation of fishy flavour.

Table 11: Variance analysis of fishy

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	3.64	9	0.40	10.62	0.0047	significant
A-xylose concentration	*0.31*	*1*	*0.31*	*8.10*	*0.0294*	
B-reaction temperature	*1.28*	*1*	*1.28*	*33.74*	*0.0011*	
C-reaction time	*0.073*	*1*	*0.073*	*1.91*	*0.2157*	
AB	*0.18*	*1*	*0.18*	*4.86*	*0.0697*	
AC	*0.046*	*1*	*0.046*	*1.21*	*0.3126*	
BC	*0.46*	*1*	*0.46*	*12.17*	*0.0130*	
A^2	*2.637E-003*	*1*	*2.637E-003*	*0.069*	*0.8012*	
B^2	*0.68*	*1*	*0.68*	*17.99*	*0.0054*	
C^2	*0.30*	*1*	*0.30*	*7.85*	*0.0311*	
Residual	0.23	6	0.038			
Lack of Fit	*0.23*	*2*	*0.11*			
Pure Error	*0.000*	*4*	*0.000*			
Cor Total	3.87	15				
Std. Dev.	0.20	R-Squared			0.9409	
Mean	1.41	Adj R-Squared			0.8523	
C.V. %	13.82	Pred R-Squared			N/A	
PRESS	N/A	Adeq Precision			9.998	

Three-dimensional and contour plot figures of interactive effects of the independent parameters on the formation of fishy flavour are shown in Figure 17 and Figure 18. It shows that the independent parameters (xylose concentration, reaction temperature and reaction time) effects on the formation of fishy flavour in the MR between xylose and FPH.

Figure 17(a) shows that the interactive effects of xylose concentration and reaction temperature on the formation of fishy flavour in the MR between xylose and FPH are curved. In the same way, From Figure 17(b) and(c), the interactive effects of xylose concentration and reaction time and the interactive effects of reaction temperature and reaction time on the formation of fishy flavour in the MR are curved too.

Increased reaction time decreased the fishy taste same did higher xylose concentration. Figure 18(a) shows that the density of contour lines along the axis of xylose concentration is higher than those along the axis of reaction temperature. That suggests the effect of xylose concentration is bigger than that of reaction temperature regarding the fishy flavour in the MR. Similar, from the Figure 18(b) and (c), conclusions can be drawn that the effect of reaction time is higher than that of xylose concentration, and the effect of reaction time is higher than that of reaction temperature.

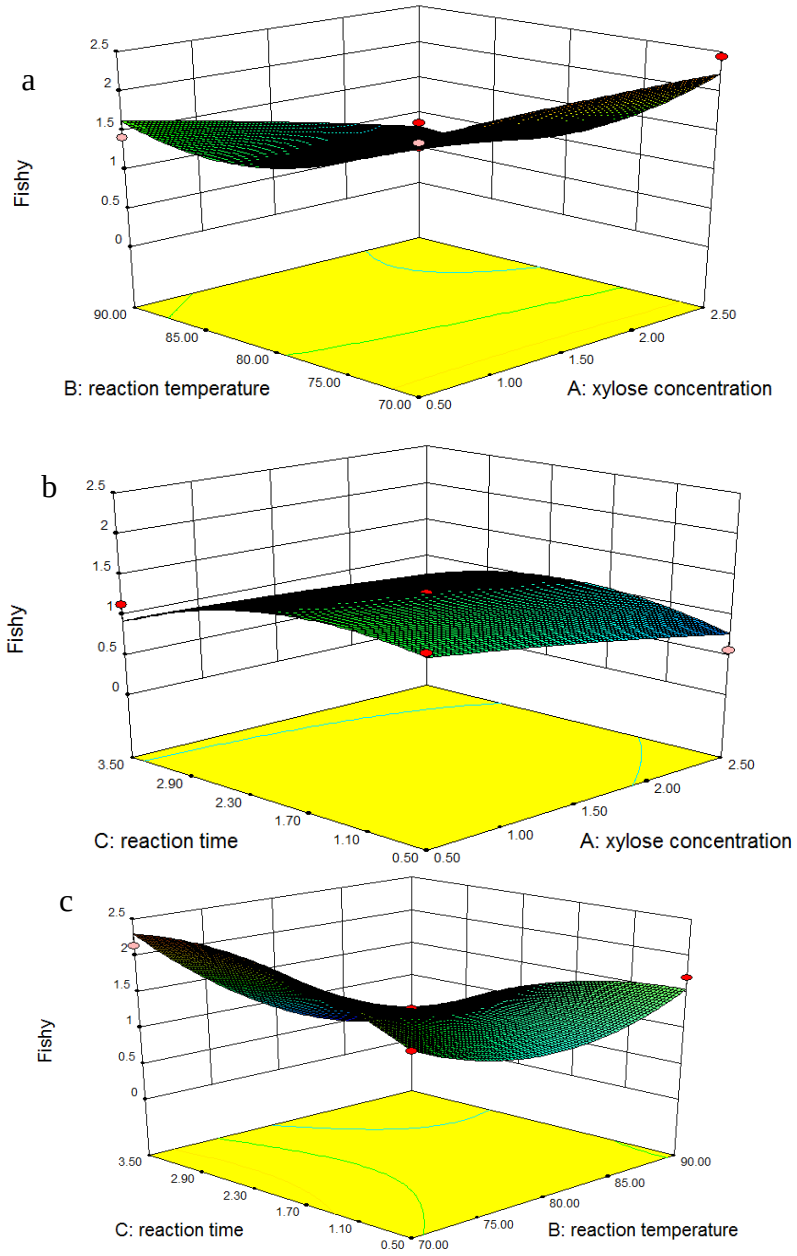

Figure 17: Three-dimensional figures of interactive effects of xylose concentration, reaction temperature and reaction time on the formation of fishy flavour (a) Interactive effects of xylose concentration and reaction temperature on the formation of fishy flavour; (b) interactive effects of xylose concentration and reaction time on the formation of fishy flavour; (c) interactive effects of reaction temperature and reaction time on the formation of fishy flavour.

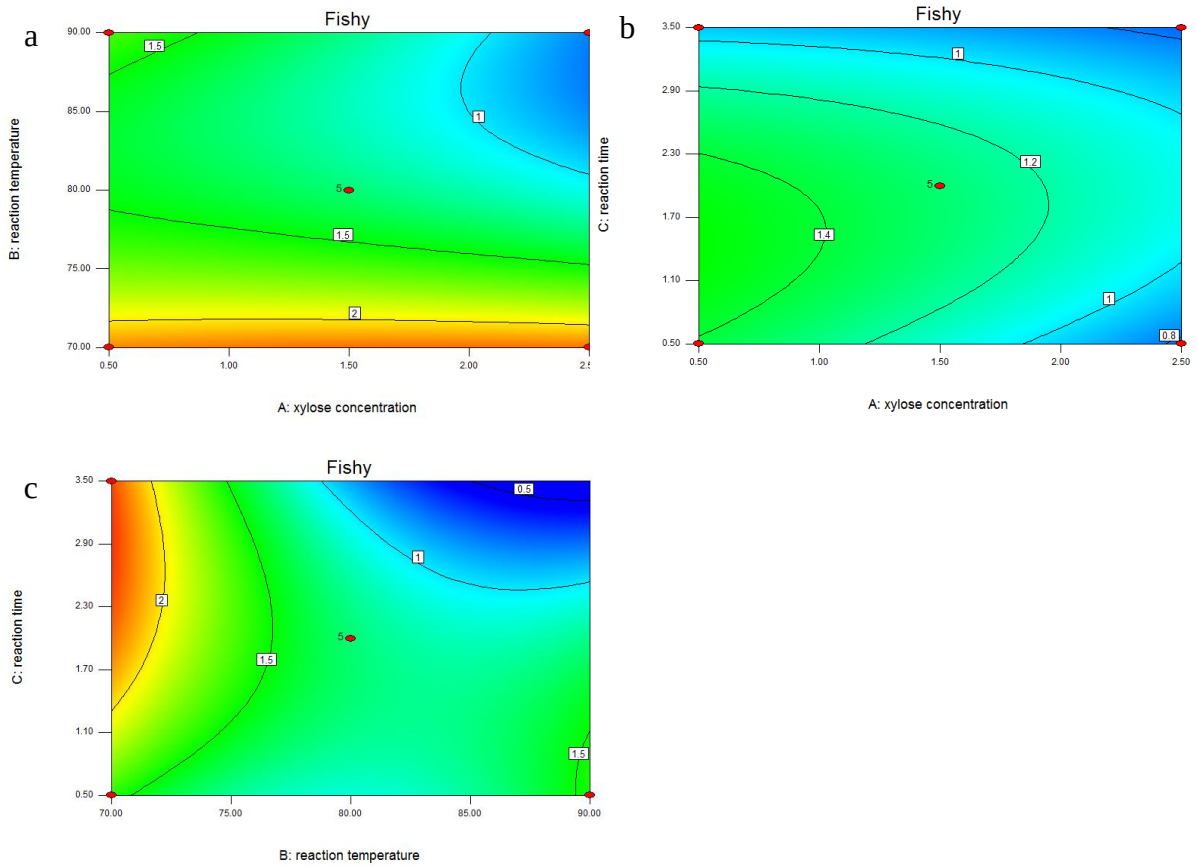

Figure 18: Contour plot figures of interactive effects of xylose concentration, reaction temperature and reaction time on the formation of fishy flavour (a) interactive effects of xylose concentration and reaction temperature on the formation of fishy flavour; (b) interactive effects of xylose concentration and reaction time on the formation of fishy flavour; (c) interactive effects of reaction temperature and reaction time on the formation of fishy flavour.

4.3.4 Response of burnt flavour formation

Table 12 shows that the formation of burnt flavour is positively related to the linear effect of xylose concentration, reaction temperature and reaction time. The quadratic terms of reaction time have a negative effect (equation (6)), and these terms significant effects on the formation of burnt flavour.

Table 12: Variance analysis of burnt

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	14.02	9	1.56	72.52	< 0.0001	significant
A-xylose concentration	*0.43*	*1*	*0.43*	*19.91*	*0.0043*	
B-reaction temperature	*4.87*	*1*	*4.87*	*226.68*	*< 0.0001*	
C-reaction time	*4.68*	*1*	*4.68*	*217.87*	*< 0.0001*	
AB	*4.900E-003*	*1*	*4.900E-003*	*0.23*	*0.6499*	
AC	*0.081*	*1*	*0.081*	*3.78*	*0.0998*	
BC	*5.281E-004*	*1*	*5.281E-004*	*0.025*	*0.8806*	
A^2	*6.939E-003*	*1*	*6.939E-003*	*0.32*	*0.5905*	
B^2	*0.022*	*1*	*0.022*	*1.01*	*0.3540*	
C^2	*1.25*	*1*	*1.25*	*58.32*	*0.0003*	
Residual	0.13	6	0.021			
Lack of Fit	*0.13*	*2*	*0.064*			
Pure Error	*0.000*	*4*	*0.000*			
Cor Total	14.15	15				
Std. Dev.	0.15	R-Squared		0.9909		
Mean	2.17	Adj R-Squared		0.9772		
C.V. %	6.75	Pred R-Squared		N/A		
PRESS	N/A	Adeq Precision		31.815		

Three-dimensional and contour plot figures of interactive effects of the independent parameters on the formation of burnt flavour are shown in the Figure19 and Figure20. They show the effect of independent parameters (xylose concentration, reaction temperature and reaction time) on the formation of burnt flavour in the MR between xylose and FPH.

Figure 19(a) shows that the interactive effects of xylose concentration and reaction temperature on the formation of burnt flavour in the MR between xylose and FPH are curved. In the same way, Figure 19(b) and(c), show the interactive effects of xylose concentration and reaction time and the interactive effects of reaction temperature and reaction time on the formation of burnt flavour in the MR are curved too.

From Figure 20(a), it very clear that the density of contour lines along the axis of xylose concentration is higher than those along the axis of reaction temperature. That suggests the effect of xylose concentration is higher than that of reaction temperature on burnt flavour in the MR. Similar, from the Figure 20 (b) and (c), conclusions can be drawn that the effect of xylose concentration is higher than that of reaction time, and the effect of reaction time is higher than that of reaction temperature.

Increased reaction time, temperature and xylose concentration increased the burnt flavour.

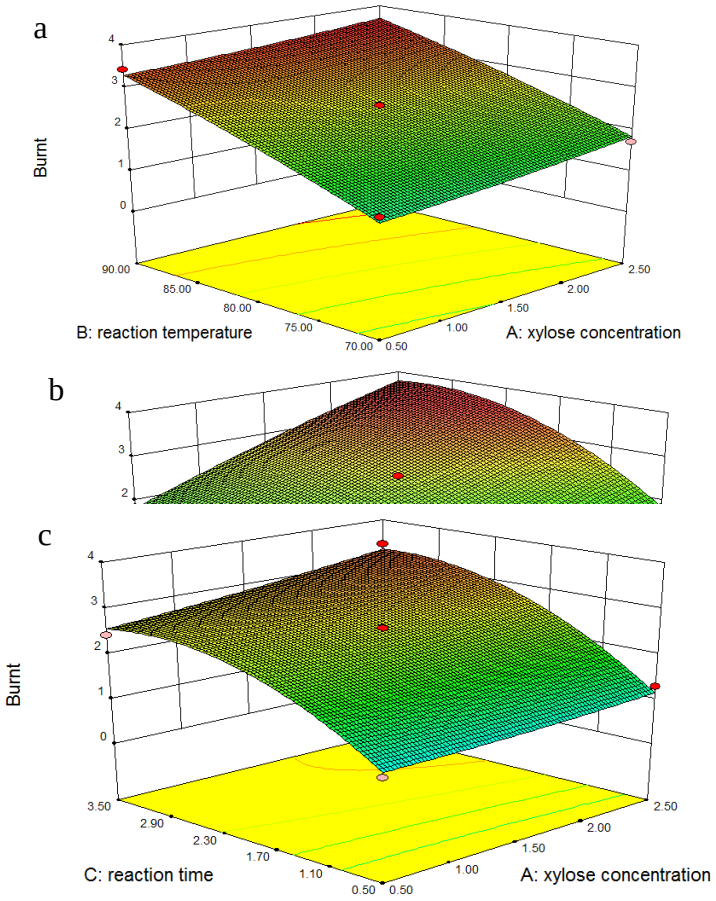

Figure 19: Three-dimensional figures of interactive effects of xylose concentration, reaction temperature and reaction time on burnt (a) interactive effects of xylose concentration and reaction temperature on burnt; (b) interactive effects of xylose concentration and reaction time on burnt; (c) interactive effects of reaction temperature and reaction time on burnt.

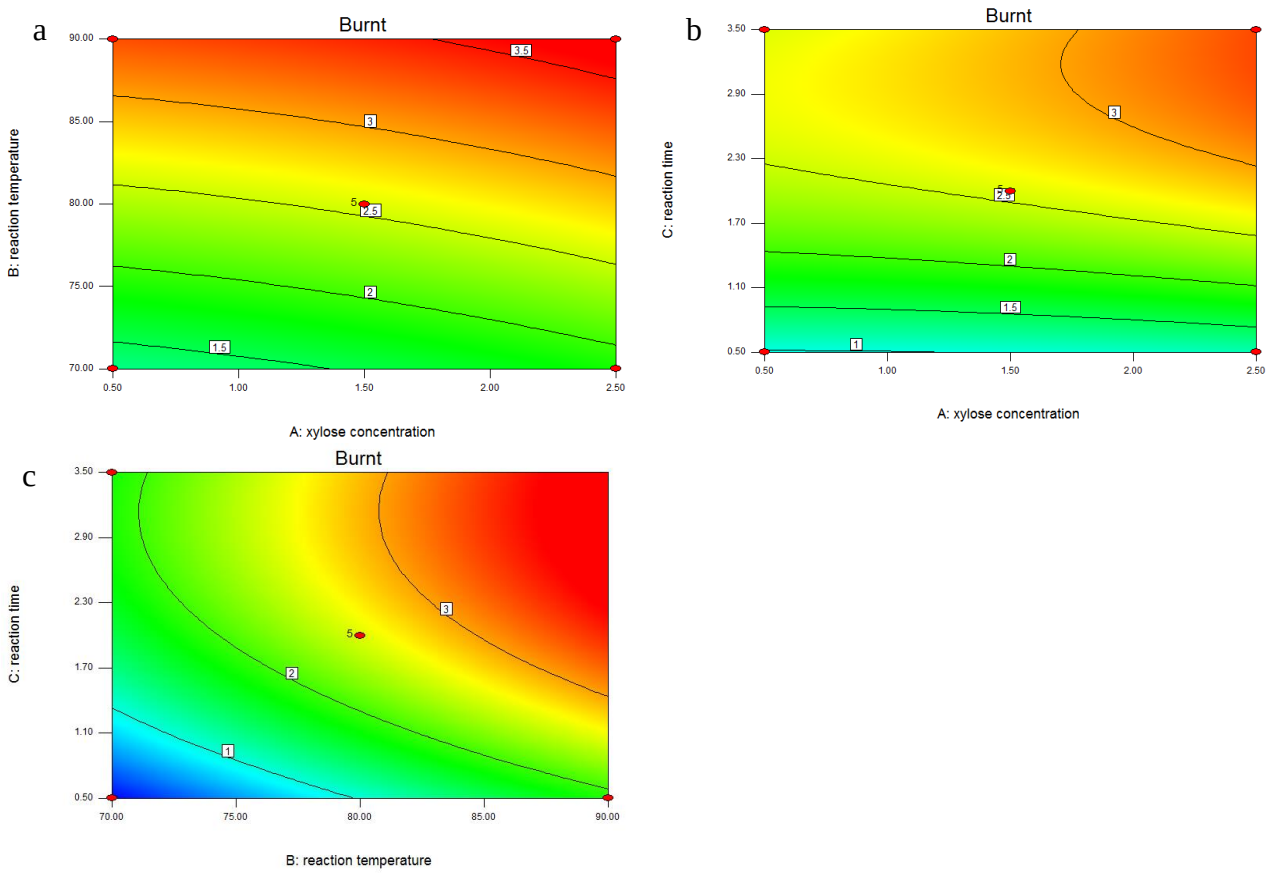

Figure 20: Contour plot figures of interactive effects of xylose concentration, reaction temperature and reaction time on burnt (a) interactive effects of xylose concentration and reaction temperature on burnt; (b) interactive effects of xylose concentration and reaction time on burnt; (c) interactive effects of reaction temperature and reaction time on burnt.

4.4 Fish sauce analog

The previous results showed that heated mixtures of FPH and xylose gave strong reaction flavours with fish sauce character. In order to see if a fish sauce analog could be produced in larger scale using results from the RSM (Ch.4.3) 3.83 kg FPH, 57.5 g xylose (1.5%) were heated for 0.93 hours at 90°C. After heating the reaction mixture was centrifuged and the sediment discarded leaving a clear supernatant.

The $yield_2$ of supernatant was 79.8% and the nitrogen recovery in the supernatant was 73.3%. The pH value of the supernatant is 6.12. And analysis of the supernatant is shown in Table 13.

Table 13: The chemical composition of the supernatant

Chemical composition	Water (%)	Solid content (%)		
		Protein (%)	Ash (%)	Salt (%)
Supernatant of MRP	81.01% ± 0.68	13.58% ± 0.28	2.85% ± 0.01	2.16% ± 0.03

The absorbance at 292nm was 0.5908 and at 420nm 0.1931. This is lower than the target value (0.2945 at 420nm).

The absorbance spectra is shown in Figure 21. The spectra are similar to the spectra obtained previously (see Figure10) indicating similar reaction between the two reaction occasions.

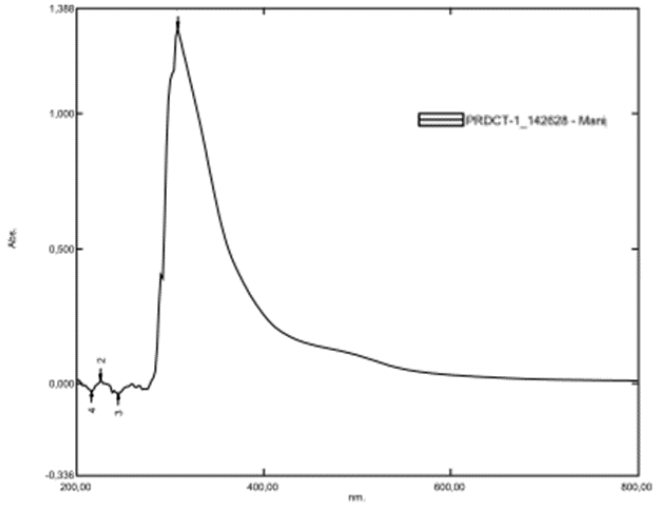

Figure 21: UV-Vis spectra of the supernatant

The colour of the supernatant was brown with slightly red-yellow colour tone. The L*a*b values are shown in Table 14 and corresponding colour pallet is below (Figure 22).

Table 14: L, a, b values of the supernatant

	Value
L	**43.58 ± 0.08**
a	**5.96 ± 0.06**
b	**8.21 ± 0.10**

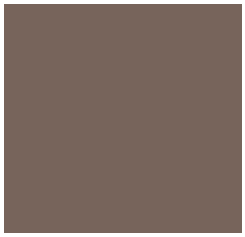

Figure 22: The colour pallet of the supernatant

Sensory analysis of the fish sauce analog is shown in Figure 23. As expected the flavour profile of the FSA (fish sauce analog) falls between those of FPH and xylose heated for 1 respectively four hours. The FSA had slightly less fishy and sweet flavour and less burnt that xylose heated for four hours and more that mixture heated for 1 hour.

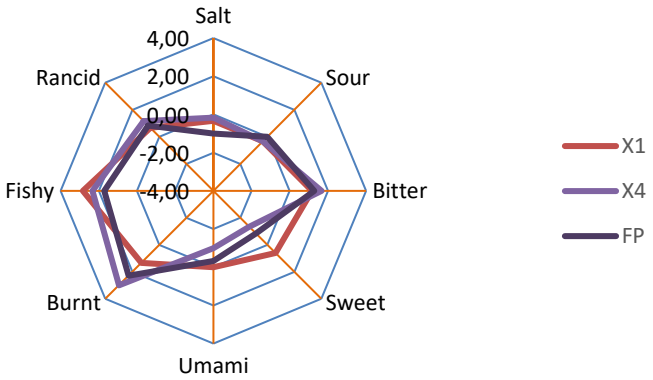

Figure 23: Comparative analysis of the sensory attributes between FSA, X1 and X4

The FSA had many characters associated with traditional fish sauces from SA- Asia including China. It had had a strong flavour, a clear fishy taste, the protein content was 13.5 % and the colour was dark amber. The FSA was however much lower in salt and thus could be a low salt alternative to traditional fish sauces. As it was low in salt the FSA must be preserved by chilling, freezing, drying or by addition of salt.

5 CONCLUSIONS AND RECOMMENDATIONS

The present study clearly demonstrated that enzyme hydrolysis of minced cod cut-off (CCHs) could be significantly modified by the Maillard reaction (MR) resulting in increased fish sauce-like flavour (e.g fishy, burnt) with low salt content at relatively low reaction temperature (<90℃) and short time (<1 hour). A fish sauce analog can be made under the optimal conditions of MR obtained by Response Surface Methodology (REM).

Traditional fish sauces are typically made in a two-year process. This study shows that this process could potentially be reduced to one or two days.

The results provide a reference for effective and practical control for MR from xylose and CCHs to enhance the formation of fish sauce-like flavour, meanwhile to achieve full utilization of cod cut-off waste and improve the added value of fishery products.

Further research work is including: using HS-SPME combined with GC-MS to identify the volatile flavour compounds and obtain the profile of product flavour, decreasing the sediment in the MRPs by utilization of enzyme.

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The University of Akureyri has provided me excellent environment and facilities to work and learn. All the staff are thanked for their valued contribution during my research work. Special thanks to Sean M. Scully and Guðný Jóhannsdóttir, for their hearty guidance, support and help in the project process.

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APPENDIX

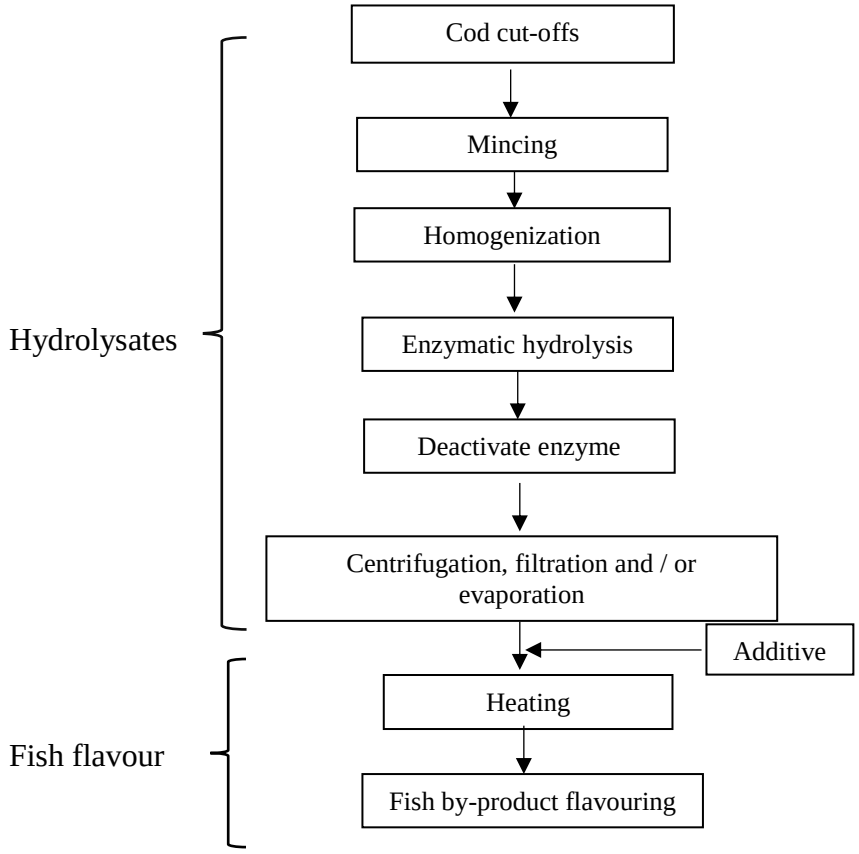

Figure 1: Processing flow diagram for production of fish flavours